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INTEGRATED SUSPENDED GROWTH BIOREACTOR SYSTEM (i-SGBR) FOR BEVERAGE WASTEWATER DENITRIFICATION AND SLUDGE DEGRADATION.

by

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INTEGRATED SUSPENDED GROWTH BIOREACTOR (i-SGBR) SYSTEM FOR BEVERAGE WASTEWATER DENITRIFICATION AND SLUDGE DEGRADATION

by

NASIRU AMINU

A Thesis

Submitted to the Postgraduate Studies Programme

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DECLARATION OF THESIS

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DEDICATION

To my mother, wife and children

As hard as my going back to acquire PhD has been for me, it has absolutely been much harder for them to cope.

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ABSTRACT

In the present study, effort has been made to assess treatability of beverage wastewater using three sequencially arranged suspended growth bioreactors, consisting of anoxic (ANX-C), aerobic (AER-C), and aerobic digester chambers (AD-C). Settler was incorported to separate sludge. Biodegradation efficiencies of pollutants such as chemical oxygen demand (COD), Total suspended solids (TSS), ammonia-nitrogen (NH_3^+-N) and nitrate nitrogen (NO_3^--N), were examined by changing organic loading rate (OLR), hydraulic retention time and internal recycle ratio. ANX-C and AER-C HRT and OLR ranges between 9.6-21 and 20-66.7 hours, and 1.34-3.85 and 0.21-0.79 kg COD/m^3 d and, respectively. AD-C was monitored for solids degradation with SRT of 10 days. Minimum bioreactor HRT resulted 95 % COD removal efficiency, with effluent clarifier (E-CLR) concentration of 69±2 mg/L. Effluent TSS concentration was observed as 41±1.8 mg/L, indicating removal efficiency of 91.1 %. Ammonia loading rate of 60.2±1.2 mg NH_3^+-N/m^3 d gave rise to E-CLR concentration and nitrification efficiency of 5.12±0.01 mg/L and 89.8±0.2%, respectively. E-CLR nitrate concentration of 18.1±0.5 mg/L was obtained, where observed nitrates concentration into ANX-C based on combined flow and effluent ANX-C were 46.4±0.9 and 0.5±0.03 mg/L, respectively. Nitrate loading rate of 1.12±0.02g NO_3^--N/d, resulted denitrification percentage and rate of 98.9±0.1 % and 252±8 mg NO_3^--N/g VSS d, respectively. AD-C achieved between 32 to 9.7 % mixed liquor volatile solids and MLSS reduction, respectively. Maximum substrate utilization rate (k), half velocity constant (Ks), growth yield co-efficient (Y), and decay coefficients (k_d) were determined from Monod's model as 2.81 d^{-1}, 979 mg sCOD/L, 0.72 mg VSS/mg sCOD, and -0.0172 d^{-1}, respectively. Maximum specific growth rate (μ_{max}) was found as 2.03 d^{-1}. Removal efficiencies dropped with reduced HRT and increase in OLR applied to bioreactor. AD-C efficiency reduced due to increase in solids loading. This study has shown applicability of integrated bioreactor to accomplish stable performance of organic matter removal and nitrification, denitrification, and sludge degradation.

ABSTRAK

Dalam kajian ini, usaha telah dibuat untuk menilai Kebolehrawatan air sisa minuman menggunakan tiga berurutan diatur bioreaktor pertumbuhan terampai, yang terdiri daripada anoxic (ANX-C), aerobik (AER-C), dan dewan pencerna aerobik (AD-C). Kecekapan pembiorosotan bahan pencemar seperti keperluan oksigen kimia (COD), jumlah pepejal terampai (TSS), ammonia-nitrogen (NH_3^+-N) dan nitrogen nitrat (NO_3-N), telah diperiksa dengan perubahan kadar permuatan organik (OLR), masa pengekalan hidraulik dan nisbah kitar semula dalaman. HRT untuk ANX-C dan AER-C adalah antara 9.6-21 dan 20-66.7 jam masing-masing. AD-C telah dioperasi sebagai sistem sub dengan SRT 10 hari untuk memantau penurunan pepejal. Pada HRT bioreactor yang minimum, kecekapan penyingkiran COD yang mencapai 95 % telah diperolehi, bersamaan dengan kepekatan efluen penjernih (E-CLR), iaitu 69±2 mg/L. Kepekatan TSS efluen telah diperhatikan sebanyak 41±1.8 mg/L, mewakili kecekapan penyingkiran sebagai 91.1 %. Kadar muatan ammonia sebanyak 60.2±1.2 mg NH_3^+-N/m^3 d menghasilkan kepekatan E-CLR dan kecekapan nitrifikasi iaitu 5.12±0.01 mg/L dan 89.8±0.2 % masing-masing. Kepekatan nitrat E-CLR sebanyak 18.1±0.5 mg/L telah diperhatikan, di mana kepekatan nitrat ke dalam ANX-C berdasarkan pengabungan aliran dan efluen ANX-C telah diperhatikan sebagai 46.4±0.9 dan 0.5±0.03 mg/L masing-masing. Kadar permuatan nitrat ke dalam ANX-C adalah 1.12±0.02g NO_3^--N/d, menyebabkan peratusan dan kadar denitrification sebanyak 98.9±0.1% dan 252±8 mg NO_3^--N/g VSS d masing-masing. AD-C mencapai antara 32 hingga 9.7% MLVSS dan pengurangan MLSS masing-masing. Kadar penggunaan substrat *(k)* maksimum, malar halaju separuh *(K_s)*, pekali hasil pertumbuhan *(Y)*, dan pekali reput *(k_d)* telah ditentukan daripada model Monod sebagai 2,81 d^{-1}, 979 mg sCOD/L, 0.72 mg VSS/mg sCOD dan -0.172 d^{-1} masing-masing. Kadar pertumbuhan tentu maksimum, *(μ_{max})* adalah didapati sebagai 2.03 d^{-1}. Kecekapan penyingkiran menurun dengan penurunan dalam HRT dan peningkatan dalam OLR. Kecekapan AD-C menurun disebabkan oleh peningkatan dalam pepejal loading. Bioreaktor bersepadu telah dicapai prestasi yang stabil dan memuaskan penyingkiran organik perkara dan penitritan, denitrification, dan degradasi enapcemar.

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NOMENCLATURE

i-SGBR	Integrated Suspended Growth Bioreactor system
ANX-C	Anoxic chamber
AER-C	Aeration chamber
AD-C	Aerobic digestion chamber
CLR	Clarifier
HRT	Hydraulic retention time
OLR	Organic loading rate
SRT	Solids retention time
IR	Internal recycle
BOD_5	Biochemical oxygen demand
COD	Chemical oxygen demand
TSS	Total suspended solids
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
I	Influent
E	Effluent
E-ANX-C	Effluent Anoxic chamber
E-AER-C	Effluent Aeration chamber
E-CLR	Effluent Clarifier
IAD	Influent Aerobic digester
EAD	Effluent Aerobic digester
I-TSS	Influent total suspended solids
E-TSS	Effluent total suspended solids
AD	Aerobic digester
EPI	Environmental Performance Index
FBI	Food and beverage industry
WWTP	Wastewater treatment plant

NOMENCLATURE Continued

ASP	Activated sludge process
UTP	Universiti Teknologi PETRONAS
BNR	Biological nitrogen removal
IWK	Indah water Konsortium
EQA	Environmental Quality Act
UASB	Upflow anaerobic sludge blanket
MBR	Membrane bioreactor
DoE	Department of Environment
WQI	Water Quality Index
SVI	Sludge volume index
pH	Potential of hydrogen
DO	Dissolved oxygen
IRC	Information Resource Center
SSV	Settled sludge volume
SBR	Sequencing batch reactor
EA	Extended aeration
F/M	Food to micro-organisms ratio
ANAMMOX	Anaerobic ammonia oxidation
SHARON	Single reactor high activity ammonium removal over nitrite
CANON	Completely autotrophic nitrogen removal over nitrite
C/N	Carbon to nitrogen ratio
RAS	Return activated sludge
MLE	Modified Ludzack-Ettinger
TP	Total phosphorus
C/N/P	Carbon/Nitrogen/Phosphorus
DC	Decentralized systems
IAWPRC	International Association on Water Pollution Research and Control
ASM	Activated sludge model
AD	Aerobic digester

HPC	Heterotrophic plate count
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
AMO	Ammonia monooxygenase
HAO	Hydroxylamine
NOB	Nitrite oxidizing bacteria
NOR	Nitrite oxidereductase
NAR	Nitrate oxidereductase
NIR	Nitrite oxidereductase
NcOR	Nitric oxidereductase
NsIR	Nitrous oxidereductase
ANB	Aerobic ammonia-oxidizing non-lithotrophic bacteria
Q	Flow rate
η_N	Nitrification efficiency
r_N	Nitrification rate
η_D	Denitrification efficiency
r_D	Denitrification rate
ALR	Ammonia loading rate
S	Influent soluble COD concentration
So	Effluent soluble COD concentration
K_s	Half-saturation constant
k	Maximum substrate utilization rate
Y	Cell yield co-efficient
K_d	Decay co-efficient
μ_{max}	Maximum specific growth
ANOVA	Analysis of variance

CHAPTER 1

INTRODUCTION

1.1 Background

Malaysia is aiming towards realization of vision 2020 to become a developed nation, through the accomplishment of its policy agenda for heavy industrialization [1]. The consistent and rapid growth of urban-industries in Malaysia has undoubtedly lead to an increase in the economic position of its citizens [2]. In spite of this, it is also indisputable that, while industrial growth is fundamental to the advancement of any country, it is likewise recognized to be a major source of pollution and resource depletion, leading to environmental degradation [3]. Upon these challenges, Malaysia has an impressive record of global headway, for being distinct as evident in the environmental performance index (EPI) report, where in 2016 it ranked 63rd out of 180 countries examined in addressing challenges, especially regarding to environmental issues [4].

Wastewater from food and beverage industries (FBI) contribute to environmental problems [5]. It is consequently imperative to tackle FBI wastewater problems [6], considering its high content of organic matter, suspended solids, and oil and grease [7, 8]. This is essential to meet the stringent environmental regulations, in the circumstances where conventional system are constraint to accomplish due to limitations in design and policy reviews. The quantity and general quality (i.e., pollutant strength, nature of constituents) of the generated wastewater have both economic and environmental consequences of its treatment and disposal [9]. Inadequate wastewater treatment can have serious ecological implications and public health [10, 11]. Discharge of poorly treated wastewater into a stream or river results in eutrophication conditions, depletion of dissolved oxygen and aquatic toxicity [12-14].

Development of improved treatment systems that can conform with the set standards are necessary [5, 15], inorder to realize effective effluent quality that can fulfil augmented production capacity, by targeting improved systems of wastewater treatment plant (WWTP) operation [16, 17]. Selection of any biological process to treat wastewater is based on the nature of wastewater and its organic matter concentration [18]. Aerobic treatment is an alternative to slower anaerobic treatment process for concentrated industrial wastewater such as FBI wastewater, and various biological treatment systems have been developed and used for the treatment of wastewater generated from FBI [18, 19].

Activated sludge process (ASP) is globally used as major biological treatment method for industrial wastewaters to effectively remove total suspended solids, organic pollutants and nitrogen [20, 21]. However, extensive application of ASP has aggravated sludge problems [22], where legislations are promulgated to control and ensure effective management of excess sludge [23]. Another major disadvantage for conventional ASP exist such as the use of large space for installation, and odorous smell. There is rising interest to curtail drawback of excess sludge problems due to operation of aerobic treatment systems, where sludge disposal accounts for 25-65 % of running cost for the operation [24]. Ideal and appropriate way to handle excess sludge related problems as post treatment, is to practically consider its reduction strategies during operation [25]. Anaerobic ponds are typically used to degrade high strength organic matter to manageable concentration, followed by supplementary treatment intermediated through aerobic systems prior to discharge into receiving streams [26]. However, anaerobic ponds followed by aerobic systems will occupy space due to segregated arrangement, and mainly if the flow rate is high. Space could be a constraint for some industries, though, the discharge could be low, which may perhaps offer an advantage to adopt an extended aeration system. These plants could be designed to treat flows as low as 0.002 million gallons per day (MGD) to as high as 0.5 MGD [27], although they could also normally treat flows between 0.01 and 0.25 MGD [28].

Biological treatment processes are very adaptive and effective in removal of organic matter and nitrogenous compounds from wastewater [29, 30]. The conventional biological nitrogen removal (BNR) is normally accomplished through two-step process,

involving denitrification which is the reduction of nitrate to nitrogen gas, and nitrification involving aerobic oxidation of ammonia to nitrate [31]. Biological nitrification/denitrification is a foremost process that has proven to be feasible, economically and technically for removal of nitrogen in decentralized systems [32-35].In this research, an inventive compact bioreactor system called integrated suspended growth bioreactor (i-SGBR) system was developed and operated as a pilot plant to treat FBI wastewater. All the treatment units are integrated into a single footprint system with internal and external recycle of biomass to transfer nitrates from aeration chamber and maintain biomass concentration in the aeration chamber. The bioreactor ascends vertically upward in concentric arrangement as a substitute of dispersing adjacently. The operation was specific to biodegradation of organic matter, removal of total nitrogen, and simultaneous reduction of excess sludge production. The proposed compact bioreactor system is targeted at small and medium size industries that discharge high strength wastewater with low discharge.

1.2 Problem statement

Wastewater from beverage industry originate from variety of several processes which comprises washing, product filing, heating or cooling and cleaning-in-place system, beverage manufacturing, and sanitizing [15, 36-38]. A great segment of the wastewater generated from FBI is highly contaminated with organic matter, dissolved solids, suspended solids, and oil & grease [14, 39-41]. Discharge of high strength wastewater coupled with increase in production line usually results due to challenges ranging from over utilized capacity and poor maintenance cultures [42, 43]. Partial treatment and discharge of wastewater into water bodies can generate various problems, including elevated BOD_5, high suspended solids, significant nitrogen impact to set in eutrophication, ecosystem interruption including aquatic toxicity, and possible exposures on health due to potential pathogens [10, 11].

In Malaysia, industries are not allowed to discharge effluent into centralized sewer systems managed by Indah Water Konsortium (IWK). Instead, they should own decentralized (on-site) wastewater treatment (WWTP) facility to treat their wastewater

as required by law [44]. The treated effluent must comply with the revised Environmental Quality Act (industrial, EQA, 1974) discharge standards [45]. These review sets more stringent discharge limits, which include requirements for standard B effluent benchmark set for BOD_5, COD, TSS and ammonia-nitrogen concentrations at 50 mg/L, 200 mg/L, 100 mg/L and 20 mg/L, respectively.

The conventional systems to treat beverage industry wastewater typically adopt anaerobic methods owing to ability in degradation of high organic strength concentrations into manageable limits that would require post treatment, energy generation potentials, and low yield of excess sludge [26]. From this perspective, anaerobic microorganisms have low growth rate, process uncertainties, low settling rate and requirement to carryout additional treatment of deleterious effluent and gases, which includes ammonium ion and hydrogen sulfide [46]. Odor problems are common with beverage anaerobic on-site wastewater treatment systems [47, 48]. This limitation makes it uneasy and unfit to collocate premises of operation. In spite of achieving high degree of degradation from anaerobic bioreactors, it is practically impossible to attain complete stabilization of organic matter [26]. Consequently, final effluent produced from anaerobic treatment contains solubilized organic matter, which can suitably be treated aerobically, in this manner indicating the capabilities of utilizing anaerobic-aerobic configuration systems [49], that can meet COD and ammonia-nitrogen limits. Conventional anaerobic-aerobic systems to treat wastewater suffer some drawbacks which include large space requirements, segregated chambers and open bioreactors connected in series [26].

Suspended and attached growth processes are regarded as the most common conventional microbial mediums employed to conduct the secondary treatment in activated sludge process systems. Suspended growth activated sludge systems are normally operated under low organic loading rate, thus, technical and operational skills are highly desirable to improve micropollutant removal, while attached growth with bio-carriers can withstand higher organic loading conditions [26, 50, 51]. Even though, this is at the expense of enormous operational liabilities, high maintenance related problems which include membrane fouling, clogging, odor, cost of chemicals, and considerable volume of excess sludge as by products goes as waste [47, 52]. Anaerobic

processes are followed by an aerobic second stage, which can utilize conventional activated sludge [53] or extended aeration process [54] to remove ammonia-nitrogen and phosphorus [8]. The improvement from conventional treatment system evolved to the use of integrated high rate up flow anaerobic sludge blanket (UASB) in combination with aerobic attached growth bioreactors, in order to overcome the disadvantages of the conventional systems [26]. These bioreactors have clear physical separation with individual treatment units, and can offer small space footprint [55-57]. Excess sludge production and management from ASP is a crucial challenge faced in aerobic WWTP's, which could possibly constitute between 25-65% of overall associated expenditure of plant's operation [24].

The anaerobic and aerobic processes alone are not capable of achieving TN limits when solely used as options for primary treatment technology [8, 58]. Incorporation of an anoxic zone within the bioreactor system is essential to meet requirements for removal of nitrate nitrogen [59, 60]. Removal of nitrate formed in aeration chamber during nitrification demands configuring compartments that are oxygen depleted (anoxic), and zones enriched with dissolved oxygen (aerobic) [55, 61]. Pollution due to release of nitrate has become more evident with excessive discharge of industrial wastewater into water intake bodies [62]. Human health damage from methaemoglobinemia syndrome are eminent, moreover nitrates are linked as precursor of nitrosamines and nitrosamides, which are possible carcinogenic compounds, leading to gastric cancer due to exceeded content in drinking water, consequently, these impending issues have attracted worldwide attention [63, 64].

This research focuses on the development and integration of a compact bioreactor system using suspended growth process to denitrify beverage wastewater. Treatments units are assembled serially and concentrically, comprising anoxic, aeration, aerobic digestion chambers, and final clarifier for settling of sludge. This concentric configuration can be achieved within vertical and slender arrangement, operating under high MLSS concentration (2000-5000 mg/L), and longer detention times to remove COD, TN, TSS, degrade excess sludge, and to achieve clarification of sludge to meet regulatory requirements prior to final effluent discharge.

1.3 Hypothesis of the research

This research attempts to highlight the research questions as follows;

1. What current on-site wastewater treatment approaches are presently used by beverage industries?

2. How capable and efficient can integrated bioreactor system using suspended growth process, with configuration of anoxic, aerobic, aerobic digester and a clarifier, be able to treat nitrogen, degrade organic matter and decrease sludge generation?

3. To what extent do individual stages of the bioreactor configurations achieve degradation of nitrogen and stabilization of organic matter from wastewater, when operated under different conditions such as organic loading rate (OLR), hydraulic retention time (HRT) and solids retention time (SRT)?

4. Can the beverage wastewater be treated by suspended growth integrated bioreactor system to meet Malaysia's Deparment of Environment discharge standards?

5. Can the relevant parameters for design of integrated bioreactor system be obtained from Modified Monod's biokinetics model?

1.4 Aim of the study

The research aims to design, develop and operate a compact biological wastewater treatment system that integrates all treatment units into a single bioreactor system that can be implemented for use by beverage industries. This bioreactor system should be able to accomplish effective wastewater treatment that can conform to regulatory discharge limits according to Environmental Quality Act (EQA, 1974) review of 2009. This will ensure that consequences due to improper wastewater treatment practices are mitigated. Safe discharge of beverage wastewater into the environment is imperative

and crucial for environmental sustainability to be rightly guaranteed. By so doing, securing the aquatic and terrestrial ecosystem can be assured towards warranting pollutants in wastewater are reduced to a level nature can effectively handle.

1.5 Objectives of the study

The objectives of this research are enumerated below:

1. To develop the concept and design a compact pilot wastewater treatment system which integrates; pre-anoxic, aeration, aerobic digestion chambers, and a clarifier into one unit.

2. To operate and evaluate performance of pilot plant (i-SGBR) in suspended growth mode to treat beverage wastewater through monitoring the system for carbon and suspended solids removal, nitrification and denitrification performance on the influence of hydraulic retention time (HRT) and internal recycle (IR).

3. To determine the microbial growth and substrate utilization bio-kinetic coefficients for extended aeration system using Monod's model over variable organic loading rates (OLR) and solids retention time (SRT).

4. To assess the bio-degradability of excess activated sludge from aerobic digestion process in the aerobic digestion chamber of i-SGBR system.

1.6 Significance of the research

The significance of performing this research was to effectively treat beverage wastewater using integrated suspended growth bioreactor (i-SGBR) system, which transforms conventional segregated treatment units into a compact wastewater treatment system. The study aims to assist beverage industries to attain DoE Malaysia industrial discharge standards, thus reducing chances of penalties due to violations and levies from enforcement authorities. Thus, compact treatment systems could be suitable

for use by small and medium industries, which are targetted for economic and "smart growth" strategies.

1.7 Scope and limitation of the study

The scope highlights development and integration of all treatment units into a stacked configuration system, assess its applicability and performance in treating raw beverage industry wastewater as feedstock. Suspended growth mode was utilized as bacteria medium for the operation of biological treatment process. Monitoring was based on removal efficiencies to evaluate treatment performance. The bioreactor chambers operated were denitrification in anoxic chamber, combined removal of carbon and nitrification in aeration chamber, and clarification in the secondary clarifier. Aerobic digestion was operated as a sub-system to degrade excess sludge. Monitoring of digested sludge was based on MLSS and MLVSS. Specific parameters monitored for sampling and testing comprise: ammonia- nitrogen (NH_3^+-N), nitrate–nitrogen (NO_3^--N), Total Kjedahl Nitrogen (TKN), five days biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solid (TSS), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), pH, dissolved oxygen (DO), temperature, heterotrophic plate count (HPC) and detection of micro fauna (metazoa and protozoa) from aeration chamber. Results of effluent quality were benchmarked with DoE Malaysia regulatory standards.

CHAPTER 2

LITERATURE REVIEW

2.1 Chapter overview

This chapter is aimed at developing the theoretical framework for this research. Important literature, publications, and reports were studied and presented. Some reputable search engine tools such as Google scholar, Scifinder Scholar, Compendex, Proquest, University Teknologi PETRONAS library facilities and services, such as Information Resource Center (IRC), text books, and other library archives were tremendously exploited, and indeed beneficial to find and retrieve important materials.

2.2 Environmental legislation for wastewater effluent discharge in Malaysia

The alternatives for the treatment and discharge of industrial effluent varies according to the legislation, and specific standards required by law in any locality. For instance, some laws allow integration of industrial wastewater with domestic sewage in the public sewerage system without pre-treatment, discharge into the public system after pre-treatment, direct discharge into receiving body after full treatment, and effluent use after full treatment [65]. However, various concerns must be factored regarding the socio-economic status, but in any regard the protection of public health and environment is ultimate goal.

In this context, Department of Environment (DoE) Malaysia being the statutory authority responsible for pollution prevention, abatement, and control, and environmental enhancement, appraised and made more stringent effluent regulations [66]. Even though, level of control is more inclined to the point sources, which explicitly highlighted effluent sources from the sewage treatment plants, manufacturing

industries, agro-allied industries and animal farms. Consequently, these limits for effluent discharge from the sewage and industries must comply with the amendment of the Environmental Quality Act (EQA, 1974) review of 2009 [45], according to SEVENTH SCHEDULE (Regulation 12), EIGHTH SCHEDULE (Regulation 13) and NINTH SCHEDULE (Regulation 14). These requirements are emphasized in Table 2.1. The discharge from industries cannot end up into public owned sewer systems. As such, adequate provision must be made for decentralized systems to treat wastewater by industries, like FBI, so as to be capable of complying with set standard regulations.

Table 2.1: Acceptable Conditions for Discharge of Industrial or Mixed Effluent of Std. A and B [67]

Parameter(mg/L)	Environmental Quality Regulations, 2009			
	Unit	Standard A	Standard B	Remark
Temperature	°C	40	40	(SEVENTH SCHEDULE, Regulation 12)
pH Value	-	6.0-9.0	5.5-9.0	
BOD_5 at 20 °C	mg/L	20	50	
Suspended Solid	mg/L	50	100	
Colour	ADMI	100	200	
Oil & Grease	mg/L	1.0	10	
Ammoniacal Nitrogen	mg/L	10	20	
COD (Industrial effluent)	mg/L	80	200	Other industries, (EIGHTH SCHEDULE, Regulation 13)
Phosphorus	mg/L	na	na	List of parameters for discharge of industrial effluent or mixed effluent which best management practice to be adopted (NINTH SCHEDULE, Regulation 14)
Nitrate - Nitrogen	mg/L	20	50	
na = not applicable ADMI = American Dye Manufacturers Institute				

The industrial effluent must comply with either DoE discharge standards A or B [45]. The standards A and B are applicable to the water catchment areas, and the non-water catchment areas, respectively. Consequently, standard A is when the water uptake (for drinking purposes) is downstream, while standard B is when the water uptake is upstream, as illustrated in Figure 2.1 [66, 68].

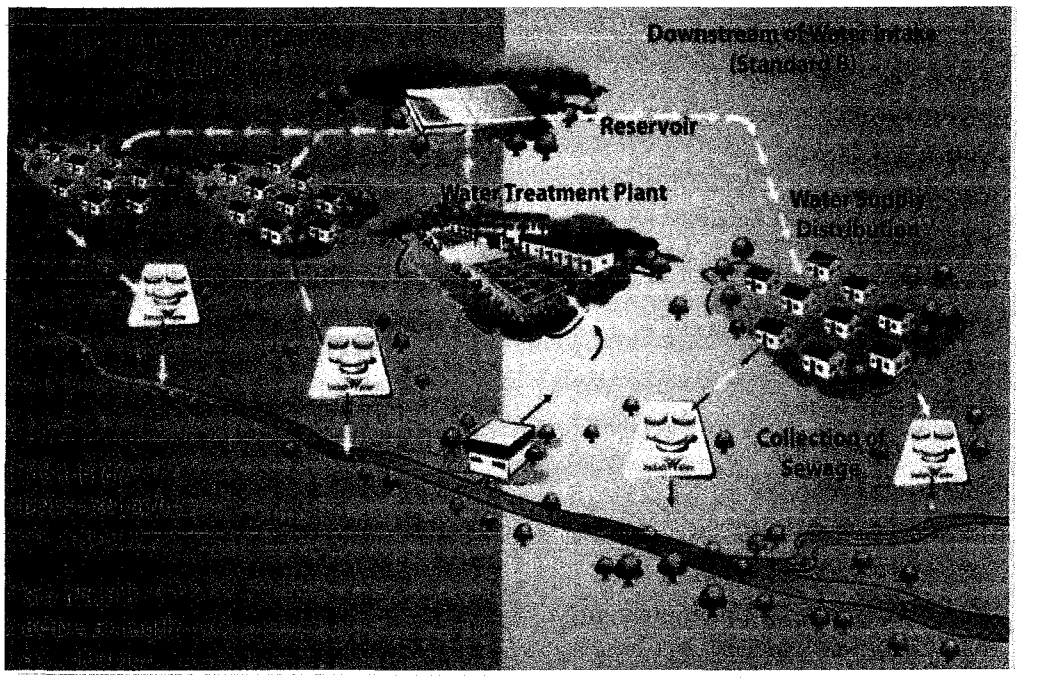


Figure 2.1: Perspective of Standards A and B [68]

The effluent discharge for organics as (BOD_5 or COD) are limited to 20 and 80 mg/L for standard A, and 50 and 200 mg/L for standard B, respectively. The effluent restrictions for ammonia-nitrogen have substantially been reduced to a minimum of 10 mg/L for standard A [45]. Nitrate and phosphorus are among the list of parameters for discharge of industrial effluent or mixed effluent, which best management practice to be adopted was declared (NINTH SCHEDULE, Regulation 14). This limitation defines the need for effective design and improved planning of wastewater treatment systems, to genuinely conform to the prerequisites of environmental protection and sustainability.

The main wastewater concessionaire in Malaysia, Indah water konsortium (IWK) is responsible for the domestic sewage treatment since 1994 and has built one of the most effective sewerage management systems [69, 70]. To improve operation and maintenance, IWK has gradually taken over sewerage systems of various sizes and types. From 1994 to 2010, more than 10, 025 systems have become public systems, and come under IWK's control, while more than 3,000 systems remain under the direct management of the owners and, thus, are classified as private systems. The IWK does not own the public facilities, but only operates and maintains them, which gives rights

to the organization of collecting sewerage charges. On the average, IWK takes over 300 treatment facilities and 1,000 km of sewer network yearly [71, 72]. However, in areas where large-scale sewerage systems are not provided, private developers will continue to construct small-scale sewerage systems [44, 73]. Since in 1980, the Malaysian parliament, passed a law, binding on the new housing developers, consisting of more than 30 units (150 population equivalent), to provide adequate facilities for sewage treatment [74].

2.3 Impact of wastewater discharges to the water environment

The introduction of organic matter into the aquatic environment results in the depletion of dissolved oxygen concentration. This occurs as a result of the process of the stabilization of the organic matter carried out by the bacteria, which utilizes the oxygen accessible in the liquid medium for respiration [75].

The release of nutrients sets in eutrophication and aquatic toxicity [12, 76]. The anthropogenic sources are the main sources of nitrate, nitrite and ammonia contamination in surface and groundwater water. The agricultural run-off, fish canning wastes, refineries, tanneries, fertilizers, and domestic wastewater are a few examples, and all can reach watercourses to cause detrimental effects to aquatic habitat, people, and animals [77]. For instance, the ammonia compound is toxic to aquatic life, as well as to the microbial growth, which increases the biological oxygen demand (BOD_5), hence decreases the dissolved oxygen (DO) concentrations within the aquatic environment. This creates "dead zones" caused by lack of DO in the water, and it is very detrimental to aquatic life [13]. Exposures to nitrate for short and long-term can cause major health complications to humans when polluted drinking water is consumed. The contamination of drinking water from short-term exposure can cause methemoglobinemia (blue baby syndrome) in infants [12]. The highly soluble nitrates and nitrites attached to the haemoglobin in the blood can result in the deficiency of oxygen in the blood, which within only a few days can be fatal if out of control [78]. On the other hand, exposures to long-term nitrates and nitrites can cause diuresis, which

is an enlarged starchy build-up and haemorrhaging of the spleen [79]. The addition of chlorine to poorly nitrified effluents, when the water is utilized downstream as potable water supply, results in disinfection by-product of serious health concern, which could be carcinogenic [80, 81].

Total nitrogen (TN) comprises both organic and inorganic (ammonium, nitrate, and nitrite) forms [82]. The influent nitrogen in wastewater can originate from human faecal matter, ground refuse, and industrial waste particularly food processing waste, whose composition are from three main sources: approximately 60 % of ammonium; 40 % of organic nitrogen, which consists of a complex mixture of organic compounds including amino acids, amino sugars, and proteins, and negligible amount of nitrate, normally less than 1 % [83]. The bacterial decomposition of protein and hydrolysis of urea readily convert the organic nitrogen in wastewater to ammonium [83, 84]. The nitrogen removal in municipal wastewater is a vital process before discharging it to natural water causes.

2.4 Biological nutrient removal

The biological nutrient removal is the removal of nitrogen and/or phosphorus through the use of microorganisms, under different environmental conditions in the treatment process [84]. The biological nutrient removal occurs through denitrification of nitrates by denitrifiers, with the nitrification of ammonia to nitrate by nitrifiers, and finally enhanced phosphorus uptake by phosphate accumulating organisms (PAO) [85]. The aerobic, anoxic and anaerobic zones form the basis of the biological nutrient removal systems [76]. The concept was initialized in the 1960's. The biological nutrient removal is the modifications of the basic ASP, and integrate the four features common to them; a flocculent slurry of microorganisms, quiescent sedimentation, settled solids recycle, and SRT control [84].

The work was done by Barnard (1975) modified development in the biological nutrient removal, where the aerobic and anoxic zones were sequenced in alternating flow, along with recycling of nitrate produced to the anoxic zone, to create cost effective

and competitive single sludge nitrogen removal system. This process is now known as the four - stage Bardenpho process. Barnard (1976) observed that biological phosphorus removal occurred in these systems if nitrate were sufficiently depleted in the initial anoxic zone, where the upstream anaerobic zone was added to the nitrogen removal system to achieve the five-stage Bardenpho process, which removes both nitrogen and phosphorus [65, 84, 85].

The ASP is versatile and a proven biological process practiced globally in the secondary WWTP's to treat both sewage and industrial wastewater. Presently, there are several modifications of the ASP to improve the quality of treatment practices, due to the stringent standards set by law [86]. However, this research covers the aerobic and anoxic zones to achieve the carbon and biological nitrogen removal.

2.5 Aerobic biological treatment

In the early years, dating back in the 1900s, the primary objective of biological wastewater treatment was to remove organic compound, colloidal and suspended solids and reduction of the pathogenic concentrations released to the receiving water bodies [84]. The removal of organic matter from wastewater requires an aerobic biological process, with the supply of DO in the aeration zone (oxic) commonly used as 2.0 mg/L [84]. The removal of organic matter during biological treatment requires adequate contact time, between wastewater and the heterotrophic microorganisms, sufficient supply of oxygen and balance of nutrients [87, 88]. Tolerable pH for the process ranges between 6.0 to 9.0 and optimum performance occurs close to neutral pH [89, 90].

Measurement parameter to access the quality of sludge settleability is the sludge volume index (SVI). The dynamic SVI may be used as a standard parameter for expressing sludge settleability, and hence dewatering prospects through simple gravity settling. The SVI is defined as the volume in milliliters occupied by one gram of dry solids after settling the MLSS for 30 minutes. In sludge analyses, first volumes of settled sludges (SSV) were measured by putting them in a graduated cylinder, and documenting the final sludge volume at the end of settling time [91]. For the ASP, SVI

values greater than 150 mL/g are indicative of filamentous bacteria in the sludge [84]. A sludge generally has good settling characteristics, if the SVI values range between 80 to 120 mL/g [92]. These values can be much lower without the presence of filamentous bacteria in the sludge, and with the improved settling due to the formation of granules [93]. A detailed SVI analysis based on extended aeration ASP and another biological process, provided SVI values between 37 to 405 mL/g [94, 95]. These results also show that the measured SVI is a function of MLSS concentration for some SVI measurement procedures. MLSS range for the previous SVI observation was in the range of 1,789 to 10,634 mg/L [96, 97].

The aerobic biological treatment involves removal of organic carbon and nitrogen from wastewater. Aerobic biological mediums to treat wastewater are classified into suspended and attached growth processes [84]. Suspended growth systems are completely mixed flocculant processes, where the microorganisms responsible for degradation of organic matter are maintained in suspension mode in the mixed liquor, while attached to an inert support medium in the case of attached growth systems, otherwise referred to as biofilm [98, 99]. The carbon removal is discussed in this section, and the nitrogen removal is explained in Section 2.7. The activated sludge processes, such as Bardenpho, pre-denitrification, post-denitrification, oxidation ditch, and sequencing batch reactor (SBR) use the principle of the suspended growth biological wastewater treatment process. These processes are explained in Section 2.8.

ASP is the most widely applied aerobic biological wastewater treatment process [100]. There are two phases to aerobic biological treatment with adequate supply of dissolved oxygen above 2.0 mg/L, the first being the mineralization of the constituents of the organics in the waste, producing carbon dioxide, water, and biomass within the aeration chamber, and secondly followed by the separation of the sludge and water after settling in the clarifier [84]. The sludge is recycled by the return activated sludge pumps, to maintain the desired concentration of the biomass in the aerobic biological reactor [84]. Due to biomass yield, part of the sludge depending on the SRT is wasted daily to maintain the MLSS concentration [65, 84].

2.5.1 Microbiology and stoichiometry

The aerobic treatment processes are carried out by very diversified and large groups of heterotrophic microorganisms, such as *Pseudomonas, Flavobacterium, Micrococcus,* and *Aeromonas* [101, 102]. This biodegradation of complex organic matter in the activated sludge system is accomplished by complex microbial ecosystem, which include aerobic bacteria, protozoa and metazoa to metabolize and flocculate large part of the organic matter within a sufficient period [103, 104].

The existence of specific types of protozoa can be used as bioindicators, thus they are interrelated to effluent quality and plant performance [105]. Protozoa play a secondary but important role in wastewater system purification [106, 107]. The protozoa in the activated sludge treatment process are categorized into four major classes comprizing: amoebae, flagellates and ciliates (free-swimming, crawling and stalked), and metazoa (rotifers and nematodes) [108] [109]. In the activated sludge biomass, over 9 % of the microorganisms comprise of ciliated protozoa and together with bacteria, they play vital role in the treatment process by removing dissolved organic matter and clarifying the treated effluent [106]. Even though, protozoa can be responsible in the essential removal of organic matter in wastewater treatment processes, their key role is to graze on bacteria [110].

The composition of organic compounds in wastewater usually consist of Carbon, Oxygen, and Hydrogen, together with Nitrogen and Sulphur in some instances, and represented by (COHNS) [84]. The COHNS is the electron donor, while oxygen is the electron acceptor. Normally, the organic matter in wastewater consist of proteins (40- 60 %), carbohydrates (25-50 %), fats and oil (8-12 %). Another major and important organic compound to be considered in wastewater is urea (CH_4N_2O), whose constituent is from urine [84, 111]. More than half of the organic substrate is oxidized during the initial biological uptake, while the remainder is assimilated in the cell tissue as new biomass, which may further be oxidized during endogenous metabolism process. Typically, according to Equation 2.1, part of the waste is oxidized to an end product to obtain energy for cell maintenance followed by synthesis of new cell tissue. Simultaneously, some of waste is converted into new cell tissue using part of energy

released during oxidation. Lastly, when organic matter is used up, the new cell begins to consume own cell tissue to obtain energy for cell maintenance, according to Equation 2.2. The term $C_5H_7NO_2$ (first proposed by Hoover and Porges, (1952), denotes cell tissue [84, 112].

Oxidation and synthesis:

$$COHNS + O_2 + nutr. \xrightarrow{bacteria} CO_2 + NH_3 + C_5H_7NO_2 + End\ products \quad (2.1)$$

Endogenous Respiration:

$$C_5H_7NO_2 + 5O_2 \xrightarrow{bacteria} 5CO_2 + 2H_2O + NH_3 + energy \quad (2.2)$$

2.6 Concept of extended aeration system

Extended aeration (EA) biological process is characterized by operational features summarized in Table 2.2 [113, 114].

Table 2.2: Basic characteristics of extended aeration [113, 114].

General item	Specific parameter	Range of values
Solids retention time (SRT)	SRT (day)	18 - 40
Hydraulic retention time (HRT)	HRT (hours)	20 – 30
F/M ratio	F/M ratio (kg BOD/kg MLVSS. d)	0.04 to 0.15
Removal Efficiency	BOD_5 (%)	93 - 98
	COD (%)	90 - 95
	Suspended solids (%)	85 - 95
	Ammonia (%)	90 - 95
	Nitrogen (%)	15 - 25
	Phosphorus (%)	10 - 20
Mixed liquor suspended solids (MLSS)	MLSS (mg/L)	2000 - 5000
Volumetric loading	Volumetric loading (kg BOD/m^3.d)	0.1– 0.3

EA systems are characterized by long SRT between 18-40 days, long HRT between 20-30 hours, low food to microorganism's ratio (F/M) ratio between 0.04-0.15 due to

endogenous respiration, high potentials for COD removal between 90-95 %, TSS between 85-95 % and ammonia-nitrogen removal above 90 % and low tendencies to treat TP between 10-20 %. EA systems could operate under high MLSS concentration ranging between 2000-5000 mg/L, and usually low volumetric loading rate ranging between 0.1-0.3 kg BOD_5/m^3 d.

2.7 Biological nitrogen removal

Numerous and dependable biological nitrogen removal (BNR) techniques are available, including Bardenpho, pre-denitrification, post-denitrification, oxidation ditch and sequencing batch reactor (SBR) [87, 115-117]. These configurations are discussed in Section 2.8. The selection of treatment process that suits low nitrogen removal (0-100 mg/L) for influent wastewaters (typically sewage and light industries), the option of biological methods are more cost effective and appropriate [30, 118]. In most countries, particularly China, about 80% of the wastewater treatment plants use the pre-denitrification [i.e., anoxic/oxic (A/O)] process for the biological nitrogen removal (BNR) [119].

The sequential biological nitrogen removal (BNR) is achieved by different types of micro-organisms. These microorganisms are nitrifiers (autotrophs) for nitrification, and denitrifiers (heterotrophs) for denitrification [31, 120]. The nitrification is the aerobic process requiring dissolved oxygen above 2.0 mg/L, in which the nitrifiers oxidize ammonia to nitrite, and then to nitrate, whereas the denitrifiers reduce the nitrate to nitrogen gas in the absence of dissolved oxygen [84]. The biological nitrogen configurations with pre-denitrification has distinct advantages for nitrogen removal, with the influent first entering the anoxic denitrification zone, the organic carbon source serves as an electron donor for denitrification, and are biodegraded by the denitrifying bacteria. This method can improve nitrogen removal efficiency and shorten the aerobic duration [116, 121]. Although, BNR systems are recognized for longer start-up and acclimation periods [122, 123], are inhibited by the effect of toxic compounds, and are frequently hindered by conditions of cold climate [124].

2.7.1 Nitrogen cycle

The biological mechanism to remove nitrogen from wastewater involves the process of nitrification and denitrification as discussed in Section 2.6. The compounds of nitrogen exist naturally with a valence ranging from -3 to +5 [31]. The conversion is facilitated by various nitrogenous species, which are interrelated by complicated interactions, in addition to diverse transformational processes [125]. The traditional pattern of the nitrogen cycle is schematically depicted in Figure 2.2 (step 2 to 6).

The nitrogen cycle is distinct by means of the nitrification (oxidation of ammonia to nitrite and further to nitrate by autotrophic biomass), denitrification (conversion of nitrate or nitrite to nitrogen gas by heterotrophic biomass), nitrogen fixation (conversion of molecular nitrogen to organic nitrogen), ammonification (death and cellular decomposition) and assimilation of ammonia to form new cells [126]. Description for the overall pattern follows the modification from previous studies [127-129], while the stoichiometry and reactions were according to [116, 130, 131].

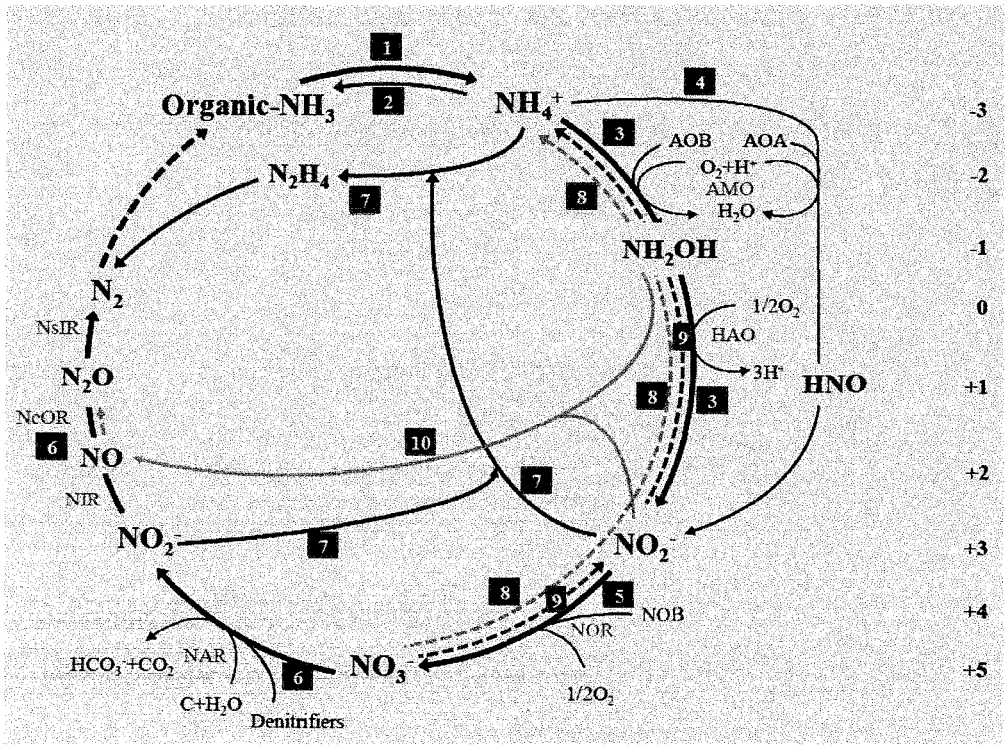


Figure 2.2: The scheme of microbial nitrogen cycle [132]

Recently, several innovative processes have evolved, resulting in advancement in biochemical pathways as described in Figure 2.2 (step 7 to 10). This comprises nitrifier denitrification, anaerobic ammonia oxidation (Anammox), the single reactor high activity ammonium removal over nitrite (SHARON) process, which is incomplete nitrification and coupling with Anammox or denitrification, and completely autotrophic nitrogen removal over nitrite (CANON) [31, 116, 117, 133]. However, some of the uniqueness for the previous innovations mentioned were reduced investments in terms of energy and oxygen requirements and lower sludge yield [31]. The general sequence of these pathways is summarized in Table 2.3.

There are numerous drawbacks to effectively operate and control the process variables like; pH, higher temperature, and low dissolved oxygen. The processes are more delicate, considering some environmental factors; ammonia concentration, DO, nitrite accumulation, low organic carbon during nitrogen removal [31, 125, 134, 135]. The adaptability to suit this innovative process to treat food and beverage industry wastewaters may not be applicable, considering the high organic content and its related unpredictability. Therefore, the traditional two-step nitrification and denitrification process remain the most effective and cost effective. Thus, widely practiced and proven biological nitrogen removal technique [136].

Table 2.3: Transformational pathway of nitrogen cycle [132]

Step	Microbial process
1	Mineralization of organic matter
2	Assimilation
3 to 6	Traditional nitrogen removal sequence via nitrification – denitrification process
3 & 4	Aerobic ammonia oxidation by ammonia oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) using ammonia monooxygenase (AMO)
5	Aerobic nitrite oxidation by nitrite oxidizing bacteria (NOB) with nitrite oxidoreductase (NOR)
6	Nitrate reduction to nitrite, nitric oxide, nitrous oxide and dinitrogen gas by denitrifiers with respective nitrogenous reductases
7	Anaerobic ammonia oxidation (Anammox)
8	Dissimilatory nitrate reduction of ammonia
9	Assimilatory nitrate reduction of ammonia
10	Aerobic deammonification and denitrification by AOB and aerobic ammonia-oxidizing non-lithotrophic bacteria (ANB)

2.7.2 Fundamentals of nitrification

The nitrification is a two-step sequential biological conversion of ammonium (NH_4^+) to nitrite (NO_2^-), and finally to nitrate (NO_3^-) under aerobic conditions by autotrophic bacteria. In the first stage, the NH_4^+ with an oxidation state of -3 is oxidized to NO_2^- with an oxidation state of +3, through NH_2OH and NOH by ammonia-oxidizing bacteria (AOB) and the lately discovered ammonia–oxidizing archaea (AOA), respectively. The enzymes involved in the process at the initial stage are ammonia monooxygenase (AMO) and hydroxylamine (HAO). While, the reaction during the second stage of the process is rapid, and NO_2^- is oxidized to NO_3^- using oxidation state of +5, by the enzymes nitrite-oxidizing bacteria (NOB) and nitrite oxidoreductase (NOR) [116, 130, 137].

2.7.2.1 Microbiology and biochemistry

The AOA and NOB are all classified under chemolithoautotrophs. They utilize carbon dioxide (CO_2) as the carbon source, drive energy from NH_4^+ and NO_2^-, and the electron acceptor is the molecular oxygen [127]. The AOB belongs to the genera *Nitrosomonas* and *Nitrosospira* (class *Betaproteobacteria*) and *Nitrosococcus* (class *Gammaproteobacteria*) [130, 138]. It was reported that AOA is related to the Kingdom *Crenarchaeota [139],* even though, the discovery was made about a single pure culture *Nitrosopumilus maritimus* [137]. The reported NOB genera are often identified, as actually capable of carrying out nitrification. They comprise *Nitrospira, Nitroga, Nitrobacter (Alphaproteobacteria), Nitrococcus (Gammaproteobacteria) and Nitrospina (Deltaproteobacteria)* [140, 141].

2.7.2.2 Stoichiometry and important factors

The stoichiometry of the nitrification process proceeds according to Equation 2.3 and Equation 2.4, with the overall process given in Equation 2.5.

$$NH_4^+ + {}^3/_2\, O_2 \xrightarrow{AOB,\ AOA} NO_2^- + 2H^+ + H_2O \quad (2.3)$$

$$NO_2^- + {}^1/_2\, O_2 \xrightarrow{NOB} NO_3^- \quad (2.4)$$

$$NH_4^+ + 2\, O_2 \xrightarrow{Nitrifiers} NO_3^- + 2H^+ + H_2O \quad (2.5)$$

The microorganisms AOB/AOA derive nearly four times additional energy according to Equation 2.3, compared to the NOB process in Equation 2.4, consequently, the yield co-efficient *(Y)* for the AOB/AOA is comparatively higher [131].

Assuming that the chemical composition of microorganisms is $C_5H_7NO_2$ (biomass) as initially proposed [112], when autotrophic cell synthesis is taken into cognisance, the global nitrification is as shown in Equation 2.6, where *Y* value of 0.17 g results from the biomass produced per gram of ammonium (NH_4^+-N) oxidized to nitrate [131, 142].

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \xrightarrow{Nitrifiers} 0.021C_5H_7O_2N + 1.041H_2O + 0.98NO_3^- + 1.88H_2CO_3 \quad (2.6)$$

The autotrophs are natural chemolithotrophic microorganisms, with a maximum specific growth rate between 0.2-0.8 day^{-1} slower, compared to 6 day^{-1} for co-existing heterotrophic bacteria, thus, being slow growers they require longer SRT [65, 143-145].

The oxidation of NH_4^+ to NO_3^- as shown in Equation 2.5 and Equation 2.6, depends essentially on the DO and alkalinity; where the theoretical DO estimation indicates that for each gram of NH_4^+ used as electron donor, 4.57 g of O_2 as electron acceptor is required, excluding considerations of biomass formation according to Equation 2.5, conversely, when the stoichiometry of nitrification considers formation of biomass according to Equation 2.6, the observation is 4.33 g of O_2 consumed for each gram for each gram of NO_3^- formed, and consumes equivalent alkalinity of 7.07 g as $CaCO_3$ g^{-1} NH_4^+ [142].

The nitrification process is affected by low DO, which restricts activity of nitrifiers, due to their constraint for oxygen half-saturation constant, where NOB have more affinity to low DO concentration, and have three times higher half-saturation constant

value, and may possibly cause nitrite accumulation by this condition [146, 147]. The DO half-saturation coefficients of 0.2–0.4 mg/L and 1.2–1.5 mg/L for AOB and NOB were reported, respectively [148, 149]. As corroborated in past researches, numerous environmental factors are found to affect nitrification, which includes pH, temperature, carbon to nitrogen ratio, and toxic compounds [84, 109, 122, 150]. These factors are explained in below.

- pH - The nitrification rate is extremely sensitive to the pH of the medium for two main reasons [151]: the inhibitory effect is substantial by both the H^+ and OH^- ions on the growth of nitrifiers, and as discussed in Section 2.7.2.2, nitrification consumes alkalinity with possibilities to cause a drop in pH. Therefore, the equilibrium between NH_3/NH_4^+ and NO_2^-/HNO_2. The formation of unionized NH_3 occurs at elevated pH, while HNO_2 forms at lower pH. The existence of unionized NH_3 and HNO_2 are identified to impede ammonium and nitrite oxidation, respectively [152]. It was reported that activity for the cultures of AOB (*nitrosomonas*) and NOB (*nitrobacter*) was optimum between pH values of 7.5 to 8.5 [153, 154]. However, later studies established nitrification was accomplished optimally between pH values of 7.0 to 9.0 [155]. Consequently, an adequate level of alkalinity needs to be maintained to ensure optimum pH, and buffer the effect of H^+ released during the transformation of NH_4^+ to NO_2^-.

- Temperature - The biochemical reactions are influenced by temperature. Hence, the growth rate of nitrifying microorganisms is sensitive to temperature [156]. Nitrification can proceed in wastewater at temperatures between 4 to 45 °C, with the nitrification rates reported to rise as a function of temperature [157, 158]. The ammonia and nitrite oxidation was considerably enhanced by increasing temperatures between 10-30 °C [150]. The experimental observations suggested that the effect of temperature on the rate of nitrification can be modeled by an Arrhenius type equation in the range of 7-30 °C (Equation 2.7) [85, 159].

$$r_{N\,(T)} = \left(r_{N\,(20)}\right)\theta^{(T-20)} \qquad (2.7)$$

Where,

T = Temperature $(^{\circ}C)$, $r_{N\,(T)}$ = Nitrification rate at temperature (T), $r_{N\,(20)}$ = Nitrification rate at 20 °C, and Θ = Temperature correction co-efficient.

- Carbon to nitrogen (C/N) ratio - The influence of C/N ratio is an important criteria in the design of BNR process for industrial wastewater treatment, Carrera et al. obtained an experimental C/N ratio for denitrification as 7.1±0.8 g COD g N^{-1}, although the stoichiometric ratio was 4.2 g COD g N^{-1}, hence this variation is related to the oxidation of organic matter in the anoxic reactor, with the oxygen of the internal recycle [122]. The heterotrophic bacteria in the ASP will strive for dominance against the nitrifiers for the DO, in which a low organic carbon concentration will be necessary for nitrification, considering higher saturation half-saturation constant for the nitrifiers [146]. The influence of C/N ratio on nitrification was investigated, where an increase in the ratio from 2 to 5 resulted in the decline of nitrification rate by 50 % [160].

- Toxic compounds - The sensitivity to toxic compounds by nitrifiers is more compared to other microorganisms, subsequently, the toxicity of heavy metals including zinc, copper, chromium, lead, cadmium, nickel, and some organic compounds such as amines, proteins, tannins, phenolics, alcohols, cyanates, ethers, carbamates and benzene have been reported to inhibit nitrification performance [130, 161].

2.7.3 Fundamentals of denitrification

The conventional heterotrophic denitrification involves the biological reduction of NO_3^- to N_2 by the reductase enzymes, with N_2 gas as the end product [162, 163]. The process is according to step 6 in Figure 2.2, and follows the sequence [84] in Equation 2.8.

$$NO_3^- \xrightarrow{Nitrate\ reductase} NO_2^- \xrightarrow{Nitrite\ reductase} NO \xrightarrow{Nitric\ oxide\ reductase} N_2O \xrightarrow{Nitrous\ oxide\ reductase} N_2 \quad (2.8)$$

2.7.3.1 Microbiology and biochemistry

There are basically two types of denitrification that occur under the anoxic condition: heterotrophic and autotrophic denitrification [164]. The heterotrophic bacteria consumes organic matter, while the autotrophic denitrification can use ammonia (Anammox) or sulphide [31, 165]. Most of the species of heterotrophic bacteria carrying out the process belong to the genera *Alcaligens, Bacillus* and *Pseudomonas* [166, 167]. Nitrate or nitrite serve as a terminal electron acceptor for the denitrifiers to oxidize organic carbon for biomass synthesis and production of energy, with denitrifiers being facultative heterotrophs require the absence of oxygen (anoxic condition) for the denitrification to progress [168]. The presence of oxygen is more preferred to utilize by denitrifiers than nitrate or nitrite as terminal electron acceptor since it provides for higher cellular growth and energy [169]. However, the oxygen concentration in the anoxic zone above 0.2 mg/L inhibits denitrification [170]. The presence of high oxygen concentration in the anoxic zone has tendency to repress the formation of NO and N_2O reductase, and in addition to low pH, together can cause the release of N_2O instead of the N_2 gas as the end product [129, 131, 171].

2.7.3.2 Stoichiometry and important factors

The denitrification can be achieved with enough organic carbon in the influent wastewater [172]. Otherwise, organic carbon is added externally if the C/N ratio of the influent is inadequate for the biological requirement [173, 174]. Hence, denitrification process needs organic carbon to carryout metabolic function and growth [175, 176]. Denitrification process is considered as an anoxic process, occurring in the absence of dissolved oxygen, and requires carbon source as terminal electron donor; typically from organic matter, organic matter produced during endogenous decay, or as exogenous source such as methanol and acetate [84, 174, 176-178]. Stoichiometry of denitrification with generic wastewater composition as biodegradable organic matter ($C_{18}H_{19}O_3N$) [34, 178] as terminal electron donor is expressed as Equation 2.9.

$$C_{18}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \qquad (2.9)$$

Equation 2.9 is the representative of heterotrophic denitrification reaction with influent wastewater as carbon source, excluding cell synthesis [84], while the stoichiometry for the overall expression for denitrification reaction when the synthesis of new cells is considered proceeds according to Equation 2.10, where the organic matter is representative of $C_{18}H_{19}O_9N$ [84, 98, 101, 179].

$$4.53NO_3^- + 0.61C_{18}H_{19}O_9N + 0.39NH_4^- + 4.15H_2CO_3 \rightarrow C_5H_7NO_2 + 2.27N_2 + 5.15HCO_3^- + 5.98CO_2 + 14H_2O \quad (2.10)$$

If external carbon source is considered for denitrification as methanol, the stoichiometry including the cell synthesis can be expressed according to Equation 2.11.

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7NO_2 + 0.47N_2 + HCO_3^- + 5.98CO_2 + 1.68H_2O \quad (2.11)$$

Where sufficient carbon sources for denitrification normally exist, control of the C/N ratio is not a major priority for the process, but factors influencing the removal efficiency of the system include; influent concentration, microbial concentration, the retention times of sludge and wastewater and reactor configuration [177]. However, the denitrification performance depends on the source of wastewater, C/N ratio [122, 177], DO [162], pH [180] and temperature [181]. Hence, the C/N ratio of the influent is essential for denitrification, and should also be sufficient to denitrify the nitrate formed in the oxic zone during nitrification. The recommended C/N ratio is between 6 to 11 for a single sludge recirculating system [182]. Carrera et al. found that the experimental COD/N ratio for denitrification was 7.1 ± 0.8 g COD/g N reduced, while the stoichiometric ratio was 4.2 g COD/g N [122]. The difference was attributed to the oxidation of organic carbon in the anoxic reactor with the oxygen in the recycle flow [183].

In Equation 2.11, almost 0.48 g of heterotrophic biomass is produced per each gram of carbon consumed for denitrification. The theoretical alkalinity produced is one equivalent for each equivalent of NO_3^- reduced to N_2. The alkalinity equates to 3.57 g

as $CaCO_3$ per gram NO_3^- reduced, which compensates for the recovery of about one half of the alkalinity destroyed during nitrification [184, 185]. Low pH and temperature impacts on denitrification efficiency [186]. The range between 7 to 8 has been reported as optimum pH for denitrification, although, the denitrifying bacteria can withstand a pH between 7 to 9 [180]. Temperature influences denitrification rates, and can be defined by the by an Arrhenius-type function like nitrification [30, 187].

2.8 Biological nitrogen removal (BNR) process configuration systems

The indication for evolution of biological processes to treat wastewater was dated back to decades ago [188]. These modifications of the activated sludge process using various configurations have been developed, and presently embraced widely for the biological treatment of industrial wastewater [189]. This is in view of the need to improve and control the effluent nitrogen discharge concentrations to receiving waters, due to increasingly stringent environmental regulations [190]. Previously, wastewater treatment systems were exclusively designed to remove only organic matter and suspended solids [84]. However, due to an escalation in industrialization and population growth, the design of wastewater treatment systems consider reduction of nutrients that cause eutrophication [84]. The focus is to adopt a standardized wastewater treatment system that can remove nitrogen in an effective and efficient manner.

ASP are flexible, reliable processes capable of removing soluble organic matter, stabilizing insoluble organic matter, and achieving a high degree of nitrification [109]. The removal of nitrogen is necessary in order to prevent discharges of high concentrations into receiving waters [85]. However, to remove total nitrogen from wastewater, nitrification and denitrification are the key conversion pathways [191]. The nitrification and denitrification processes have been explained in Sections 2.7.2 and 2.7.3, respectively.

Thus, consideration from foregoing discussion steered the necessity for BNR systems. The BNR is an improvement of the basic ASP and is notable by the separations of the bioreactor into distinct biochemical processes [85]. The BNR systems remove

considerable amounts of nitrogen from wastewater [192]. The supplementary addition of chemical may also be required, subject to the degree of removal to be achieved [85].

The single sludge biological nitrogen processes are grouped according to whether the anoxic zone is located upstream (preanoxic) or downstream (postanoxic) of the aerobic nitrification zone [84], (Figure 2.3 and Figure 2.4), and the microbial activity could be mediated by either suspended or attached growth processes [10, 99].

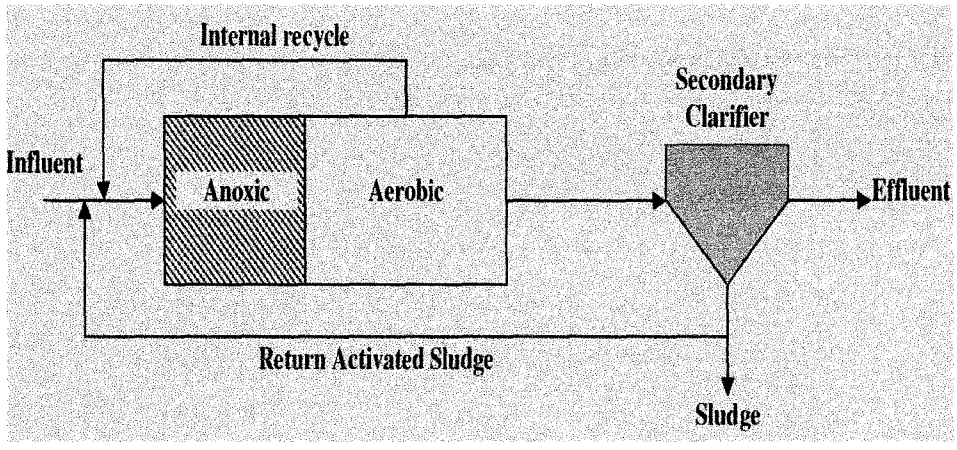


Figure 2.3: Modified Ludzak Ettinger configuration process [84]

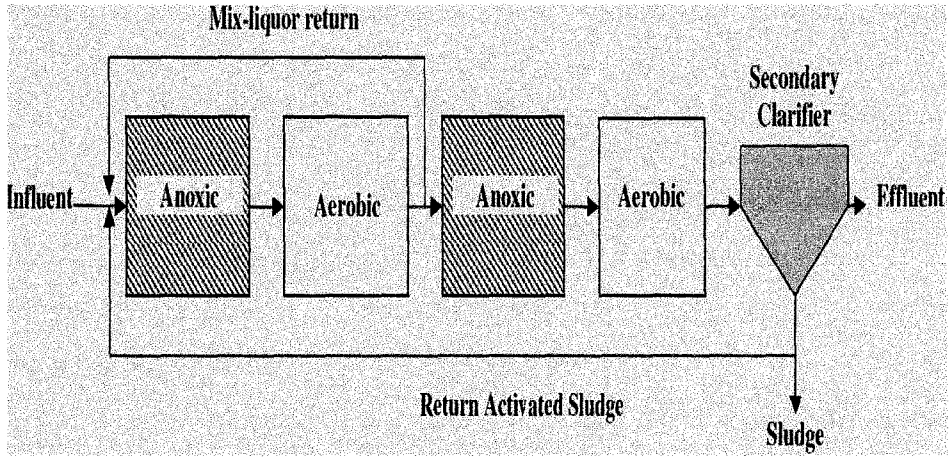


Figure 2.4: Four-stage Bardenpho configuration process [84]

Typically, the internal recirculation of nitrate in the mixed liquor produced in the aerobic reactor to the preanoxic zone facilitates the utilization and simultaneous removal of the readily biodegradable substrate [122, 193]. The recycle of the return activated sludge (RAS) from the clarifier underflow to the anoxic/aerobic zones serves

to maintain the concentration of biomass within the system, and ensure adequate contact between biomass and substrate [194]. The preanoxic zone utilizes raw wastewater as a carbon source for denitrification, while postanoxic uses the carbon source for endogenous denitrification [84]. Larger postanoxic volumes are often required since denitrification occurred by utilization of endogenous carbon source [85]. The addition of external carbon sources (e.g., methanol and acetate) to enhance denitrification rates is commonly practical to the denitrifying activated sludge, in the event that the influent is deficient of readily biodegradable carbon source to achieve the required nitrate reduction [174].

Pre-denitrification bioreactor configurations such as MLE process (Table 2.4) are normally adopted to utilize the biodegradable organic matter from influent, with tendencies to generate higher denitrification rates and resulting in lower reactor volumes [195, 196]. The removal efficiency of nitrate could be limited to range of between 60-90 % [165, 197]. However, the recycle of nitrate rich mixed liquor with sufficient dissolved oxygen concentration from aerobic zone practically restrict denitrifiers activity [162]. Due to this limitation of internal recycle, second anoxic chamber is often required as post anoxic denitrification bioreactor to accomodate the residual nitrate that was unable to be denitrified from first anoxic chamber, as well as from aerobic chamber. The four-stage Bardenpho process (Table 2.4) is a usual configuration for this case, where aerobic bioreactor is placed in between the upstream and downstream anoxic bioreactors [178]. The requirement of an additional post denitrification bioreactor for nitrogen removal process results in higher contruction, operational cost, and need for availability of exogenous carbon source.

The choice to select specific process of biological nitrogen removal process depends on site distinctiveness, existing processes, and equipment, as well as desired treatment efficiency to be achieved [84]. An assessment of the suspended growth biological nutrient removal processes is presented in Table 2.4, based on the review from [65, 84, 178, 188, 191, 198]. These process configurations are single sludge because the microbial consortia are dominant for each medium to facilitate the removal of organics, nitrification and denitrification processes [198].

Table 2.4: Common suspended growth biological processes for nutrient removal

Process	Description	Advantages	Limitations
Ludzack-Ettinger process [65, 84]	1962: Ludzack & Ettinger designed first BNR pre-denitrification reactor, with preanoxic process upstream followed by aerobic zone. Denitrification limited only by the RAS ratio.	Raw influent wastewater serves as the electron donor fed into the preanoxic zone with nitrate from RAS recycle as an electron acceptor.	The denitrification efficiency depends on the nitrate recycle from RAS, produced in the aerobic zone. High tendencies of rising sludge in the clarifier.
Modified-Ludzack Ettinger (MLE) process [65, 84, 178, 188, 191]	1973: Modification of Ludzack-Ettinger process (Figure 2.3), to include an internal mixed liquor recycle (MLR), which recycles the nitrate to upstream preanoxic zone for denitrification from the aerobic zone. 5-8 mg/L effluent TN attainable.	Saves energy, with COD, reduced before entering the aerobic zone, alkalinity is produced before nitrification, internal recycle independent of RAS which offers better control of the nitrate, smaller requirements for anoxic volumes to achieve high denitrification efficiencies.	The capability to achieve substantial nitrogen removal depends on the MLR. A portion of nitrate unable to return to preanoxic zone is discharged from the clarifier. Entails careful DO control for the transfer of the MLR.
Four-stage Bardenpho Process [65, 84, 178, 188, 191]	1975: Comprises both preanoxic and post-denitrification (Figure 2.4), with MLR from the first downstream aerobic zone only, while the nitrate from the second aerobic zone downstream of postanoxic is denitrified by either endogenous or external carbon source, and residual nitrate in clarifier returned to the preanoxic by RAS.	The residual nitrate that unable to be returned to preanoxic is denitrified in the second postanoxic zone. Capable of achieving effluent TN levels of less than 3 mg/L. Phosphorus removal possible due to alternating anoxic-aerobic sequence. The second aerobic enhances sludge settle ability prior to entering clarifier, due stripping off of nitrogen gas.	Large footprint due to the requirement for the number of reactors, and higher capital cost.

Table 2.4: Common suspended growth biological processes for nutrient removal cont.

Process	Description	Advantages	Limitations
Anaerobic/Anoxic/ Aerobic process (A^2/O) [65, 84, 178, 188, 191]	Imitate the MLE, with addition of anaerobic zone upstream of the preanoxic zone, and the RAS is returned to the anaerobic zone instead of the preanoxic zone.	Capable of simultaneous nitrate and biological phosphorus removal (BPR).	The addition of nitrate to the anaerobic zone from RAS impairs the process of BPR.
SBR [65, 84, 178, 188, 191, 198]	Five step treatment process; fill, react, settle, decant, and idle, performed in a time sequence batch mode. The process involves incorporation of equalization, aerobic, anoxic reactions and final clarification to settle the biomass.	The process performed in a single basic therefore offers a small footprint, and saves the cost of construction, MLSS cannot be washed-out by hydraulic surges. The different activities are optimized in time-phased cyclic batch mode. Effluent nitrate-nitrogen between than 5-8 mg/L can be achieved.	Redundant units are required for operational reliability, requires more sophistication and requisite maintenance demands, more complex process design, and effluent quality determine by decanting facility.
Oxidation ditch [65, 84, 178, 188, 191, 198]	The flow is continuous within a looped system, with the cyclic movement of the wastewater through aerated and non-aerated zones in a time phase sequence.	Operate as a complete mix with almost uniform MLSS and DO in the process, resistant to load variation with less impact on effluent quality. TN less than 10 mg/L possible.	High control expertise is essential, large footprint requirement to implement, tendencies of high effluent suspended solids.

2.9 Aerobic digestion of sludge

Sludge can be stabilized either by aerobic or anaerobic digestion [199]. The aerobic digestion is an extension of ASP through process of endogenous metabolism conditions [85]. The process for aerobic digestion begins with the oxidation of the biodegradable organic matter (active biomass) to carbon dioxide and water, and the organic nitrogen is mineralized to ammonia by the aid of heterotrophic bacteria. Subsequently, with the supply of sufficient DO and adequate alkalinity, the ammonia is further oxidized by the process of nitrification to nitrate-nitrogen, where the alkalinity is destroyed with resulting drop in the pH [200]. Alternatively, the condition for anaerobic digestion results in the conversion of the biomass to methane gas, carbon dioxide, and ammonia, and low molecular weight fatty acids [201]. The constraints normally encountered with these two processes include high demand for energy in aerobic digestion, the process instability for both processes, and high capital cost for anaerobic digestion [84, 202]. Although, non-biodegradable part of the particulate matter in the influent remains unchanged. Though its contents will be included in the solids digested. During the process of aerobic digestion, MLVSS and MLSS are destroyed [85, 203, 204].

In the past, the common practice was to dispose of sludge in landfills, incineration, and agricultural use [21]. Incineration reduces the volume of solids by almost 95 %, although it requires expensive machinery and consumes non-renewable resources, and has an undesirable public perception [115]. The utilization of sludge as fertilizer adds to an economic value, however, it may require long haulage distance, sludge may contain heavy metals [115], and trace organic chemicals that are possibly toxic [205].This portends the risk of food security. Therefore, sludge minimization is largely a better alternative than post-treatment [25].

Ros et al. studied aerobic degradation and stabilization of waste activated sludge under thermophilic condition in a WWTP [206]. The major measured parameters determined for reduction of organic fraction was volatile solids. Among the goals of the experiment was to determine the optimum operating temperature from 20, 37 40, 45, 50 and 55 °C. The suitable temperature for the aerobic digestion was determined as

50 °C. The other part of the experiment analysed degradation of volatile solids on a contineous flow process at temperature of 50 °C. Volatile solids reduction of 29.7 % and 48.2 % were achieved for a 5 and 10 days retention times, respectively.

Bernard et al. carried out research to find the feasibility of aerobic digestion on pharmaceutical and domestic sludges in order to determine the optimum operational conditions for aerobic digestion and monitor the requirement for pH stabilization [207]. The concentrations for samples tested to represent pharmaceutical and domestic sludge ranges from 1,500-11,000 mg/L and from 4,500-22,000 mg/L, respectively. Some of the parameters monitored include MLSS and MLVSS. The reductions achieved in MLSS and MLVSS were higher for domestic sludge than for the pharmaceutical sludge. pH stabilization at 6.5 improved the sludge characteristics with significant better supernanant quality and better sludge filterability. There were no recorded odor or or foaming problems. The performance regarding the aerobic digestion achieved between 42-53 % and 53-64 % MLSS and MLVSS reduction, respectively.

2.9.1 Stoichiometry and important factors

Once the culture of aerobic heterotrophic microorganisms is in any medium containing organic material, degradation of the organic matter will proceed until all the organic matter is consumed, and part of the organic matter removed is utilized for the cell synthesis of new microorganisms, with resulting increase in biomass. Product of oxidation for the remaining materials is carbon dioxide, water, and soluble inert matter, providing energy for the synthesis, metabolism and important maintenance functions of the microorganisms. When the external organic carbon source has been exhausted, endogenous respiration sets in, where cellular material is oxidized to fulfill the energy need to survive [85, 204].

In the conventional aerobic digestion design process, where sludge to an aerated basin is added and removed intermittently, usually once per day, their hydraulic retention time (HRT) for that period equals to the SRT [85]. The biomass quantity will

decrease subject to extended retention of such conditions, hence the remaining biomass will exist at low energy, biologically stable, relatively inert and safe for disposal [204].

The sludge minimization could be achieved through alternating redox conditions (cyclic aerobic and anoxic regimes) [21, 144]. The controlling parameters such as increasing the SRT, DO concentration can yield negligible enhancement, but may impact to increase the cost of plant operation [23]. The sludge disintegration technique considerably reduces sludge production but entails high capital investment and maintenance cost [144].

There are two basic steps that describe aerobic digestion; first is the conventional oxidation of the biodegradable organic matter, then secondly endogenous respiration in which the cellular material is oxidized [204]. These processes are expressed in Equation 2.12 and Equation 2.13.

$$Organic\ matter + NH_4^+ + O_2 \rightarrow active\ cellular\ material + CO_2 + H_2O \quad (2.12)$$

$$Active\ cellular\ material + O_2 \xrightarrow{bacteria} dig.sludge + CO_2 + H_2O + NO_3^- \quad (2.13)$$

Equation 2.12 is the initial process in the extended aeration process, where the oxidation of the organic matter (influent wastewater) yields cellular material. Equation 2.13 is representative of endogenous respiration process, and the main reaction signifying the aerobic digestion systems. The aerobic digestion in which the sludge mass is reduced is only recommended for excess activated sludge [206].

Using the formula $C_5H_7NO_2$ to symbolize mass of a typical cell of microorganism [112, 208], the stoichiometry for the aerobic digestion can be expressed according to Equation 2.14 and Equation 2.15.

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 + energy \quad (2.14)$$

$$C_5H_7NO_2 + 7O_2 \rightarrow 5CO_2 + 3H_2O + NO_3^- + H^+ + energy \quad (2.15)$$

Equation 2.14 represents the system inhibiting nitrification, where nitrogen is released in the form of ammonium. While, for a system in which nitrification occurs, is expressed by Equation 2.15. The theoretical oxygen demand in Equation 2.14 is 1.42 kg oxygen per kg of cell mass digested, while the equivalent theoretical oxygen demand according to Equation 2.15 is 1.98 kg oxygen per kg cell mass digested [204]. The oxygen requirement to achieve nitrification is comparatively higher than without nitrification, due reliance on factors such as operating temperature and SRT [84]. Aerobic digestion can occur at an ambient temperature to thermophilic temperature range [204]. The optimal temperature of 22-30 °C is key to achieve aerobic digestion and nitrification, with a supply of DO between 10-30 mg/L [204].

2.10 Influent wastewater requirements for biological treatment process

Industrial wastewater are highly variable in composition, for instance, products of brewing are deficient in nitrogen and phosphate [88]. Hence, industrial wastewater may need to be stabilized to meet influent deficiency in nitrogen, phosphorus and trace elements, so as to satisfactory meet up the balanced carbon, nitrogen and phosphorus (C/N/P) ratio to accomplish effective treatment needs [209]. Various C/N/P ratios from previous researches conducted using biological treatment are presented in Table 2.5.

Table 2.5: Influent C/N/P ratios and treatment performance for various systems

Influent C/N/P ratio	Process	BIO-R	CLR	HRT (h)	SRT (d)	TN remov. (%)	TP remov. (%)	Ref.
470/31/15	A^2O	3	1	8.7	15	72	-	[193]
300/50/4	AOA	3	1	6	20	90	99	[87]
750/75/26	UCT	3	1	24	10	78.2	48.8	[210]
178/74/5	Modified A^2O	4	1	9.3	15-20	75	98	[211]
220/53/5	AO/ IVMBR	2	-	6.7	30	74.7	22.2	[212]

Notes: BIO-R= Bioreactor,A^2O= Anaerobic, Anoxic and aerobic reactor, AOA= Anaerobic, Aerobic and Anoxic reactor, AO/IVMBR= Anoxic Aerobic/Integrated Vertical Membrane Bioreactor

Influent C/N/P ratio for wastewater should normally be above 100/5/1 to accomplish effective biological treatment process [87, 193, 209-211, 213, 214]. C/N/P

ratios indicated for respective biological treatment configurations were above the minimum essential to accomplish treatment objectives.

Wastewater from FBI generally vary on the type of product being processed, process and equipment used, although common characteristic is generally the strong organic content [6]. Food-processing wastewater can be characterized as non-toxic because its content have few hazardous and persistent compounds [215, 216]. This industry typically consumes large quantity of water for processing of products [19].

2.11 Categorization of food processing & beverage wastewater

The wastewater generated due to different activities results in organic pollution, which is typically collected and treated in a decentralized (DC) treatment facilities [217]. The food and beverage industry includes various subsectors targeting at manufacturing different types of products, and includes the following sub-sectors: processing and preserving of meat, and production of meat products, processing and preserving of fish, crustaceans and mollusks, processing and preserving of fruit and vegetables, manufacture of vegetable and animal oils and fats, manufacture of dairy products, manufacture of grain mill products, starches and starch products, manufacture of bakery and farinaceous products, manufacture of other food products, and manufacture of prepared animal feeds. While, the beverage industry include processes such as distilling, rectifying, and blending of spirits, manufacture of wine from grape, manufacture of cider and other fruit wines, manufacture of other non-distilled fermented beverages, manufacture of beer, manufacture of malt, and manufacture of soft drinks; production of mineral waters and other bottled waters [218].

The FBI are major consumers of water, as much as 10 to 12 tons of water per ton of product - or even more [219, 220]. Food-processing wastewater can be characterized as non-toxic, because it contains few hazardous and persistent compounds. With exception of some toxic cleaning products, wastewater from food-processing facilities is organic and can be treated by conventional biological technologies [215]. Food processing can be divided into four major sectors including fruit and vegetables; meat, poultry, and

seafood; beverage and bottling; and dairy operations [6, 221]. All of these sectors typically consume a large quantity of water for the processing of food product [19].

The composition and concentration of different food processing industry wastewaters differ from low (wash water from sugar mill or dairy effluents) to high strength substrates (cheese, winery and olive mill wastewaters), mainly in terms of organic matter, acids, proteins, aromatic compounds, available nutrients[5, 222, 223]. The main parameters of food industries wastewater, such as total solids (TS), total nitrogen (TN), total phosphorus (TP) and biochemical and chemical oxygen demand (BOD and COD), respectively are given in Table 2.6.

Table 2.6: Characteristics of typical food processing industry wastewater.

Industry	TS (mg/L)	TP (mg/L)	TN (mg/L)	BOD (mg/L)	COD (mg/L)	Ref.
Food processing	-	3	50	600-4,000	1,000-8,000	[224]
Dairy	1,100-1,600	-	-	800-1,000	1,400-2,500	[225]
Corn milling	650	125	174	3,000	4,850	[226]
Potato chips	5,000	100	250	5,000	6,000	[227]
Dairy	250-2,750	-	10-90	650-6,250	400-15,200	[228]
Fruit juice	-	10.2	58.2	3,134	5,157	[41]
Beverage	-	-	-	1,800	1,000	[52]

Notes: TS = Total solids, TP = Total phosphorus, TN = Total nitrogen, BOD = Biochemical oxygen demand and COD = Chemical oxygen demand. All units in mg/L

2.12 Wastewater treatment approaches for food and beverage industries

The technologies to treat food and beverage industry wastewater consistent to its composition are commonly categorized into biological, chemical, and physical methods [229]. These systems can be combined to operate hybrid process for achievement of specific goals. However, each of these technologies has its distinctive advantages and disadvantages [230, 231]. Hence, selection of wastewater treatment technology by any industrial sector broadly depends on parameters such as [232].

1. Characteristics of the waste water including its quantity (vol. in m^3/d)

and quality (COD, BOD, pH, TSS, TDS etc).

2. Mode of final discharge.
3. Area available for the effluent treatment plant.
4. Budget for effluent treatment plant.
5. Operation of the production unit (seasonal or yearly).
6. Future expansion program.

a. **Biological methods**: The fundamental applications of biological processes is to achieve removal of carbonaceous organic material (BOD), removal of total nitrogen, stabilization, and phosphorous removal. It is expedient to categorize biological approaches as aerobic or anaerobic (anoxic and anaerobic), which have been described earlier in Section 2.5 and Section 2.7. Aerobic biological processes are commonly achieving high degree of treatment efficiency, while anaerobic uses the concept of resource recovery and utilization with achieving objective of pollution control. Most wastewaters containing biodegradable connstituents with BOD/COD ratio of 0.5 or greater can be easily treated through biological process [84].

b. **Chemical methods**: Chemical treatment can be applied for removing constituents and pollutants through the following:
    1. Producing of insoluble solids and gas,
    2. producing coagulation of a colloidal suspension,
    3. producing biological degradable substances from nonbiodegradable,
    4. destroying or deactivating chelating agents, and
    5. producing non objectionable substances that can be easily removed.

c. **Physical methods**: This treatment accomplish the removal of substances by use of naturally occurring forces, such as gravity, electrical attraction, and van der Waal forces, as well as physical barriers. Physical methods of wastewater treatment include sedimentation, floatation, and adsorption, as well as barriers such as bar racks, screens, deep bed filters, membranes, electro dialysis and ion exchange [233].

2.12.1.1 Anaerobic and aerobic treatment for food and beverage industry wastewater

Anaerobic digestion technology has been used in a wide application for treatment of industrial wastewaters containing high organic matter content, including dairy wastewater [234], cheese whey wastewater [235], distillery spent wash water [236], starch wastewater [237], and slaughter house wastewater [238]. The up-flow anaerobic sludge bed (UASB) reactor technology is considered a breakthrough in the development and application of anaerobic high-rate technology for industrial wastewater, particularly for wastewaters coming from food-processing industries [41]. Difficulties with UASB reactor treating wastewater results from washout of biomass which deteriorates the effluent quality [239]. Effluent of the anaerobic reactors generally do not comply with regulatory standards for discharge into receiving water bodies, hence, post-treatment is required [41].

Anaerobic and aerobic processes can both be used to treat food and beverage industry wastewater [240]. The former involves degradation of complex organic wastes into methane gas, carbon di oxide and water through hydrolysis, acidogenesis including acetogenesis and methanogenesis in the absence of oxygen, while the latter involves application of dissolved oxygen by aerobes to degrade the organic matter with by-products as biomass, carbon di oxide and water as discussed in Section 2.5.1. Table 2.7 shows comparison of aerobic and anaerobic treatment [26, 229].

Table 2.7: Opportunities and limitations of aerobic and anaerobic treatment

Feature	Aerobic	Anaerobic
Organic removal efficiency	High	High
Effluent quality	Excellent	Moderate to poor
Organic loading rate	Moderate	High
Sludge production	High	Low
Nutrient requirement	High	Low
Alkalinity requirement	High	Low
Energy requirement	High	Low to moderate
Temperature sensitivity	Low	High
Start up time	2-4 weeks	2-4 months
Odor	Less odors	Potential odors
Bioenergy recovery	No	Yes
Mode of treatment	Total	Essentially pretreatment

Opportunities and limitations of aerobic and anaerobic treatment methods are highlighted. Aerobic biological treatment processes are normally used when high degree of effluent quality is desired, whereas anaerobic treatment effluent serves as pretreatment and anaerobic treatment can be based on concept of biotechnology for resource recovery and utilization [85]. Frostel [241] and Cervantes et al. [242] have identified the benefits of anaerobic and aerobic treatment processes of wastewater as follows:

1. High potentials of resource recovery and utilization by removing most of the organic pollutants and converting them into biogas and other gaseous by-products.
2. Achieving high degree of treatment efficiency, through provision of aerobic post-treatment which polishes the anaerobic effluent. The aerobic treatment serves to stabilizes the fluctuations in the anaerobic effluent quality.
3. Through digesting excess sludge form aerobic process anaerobically, less disposal of sludge and minimum stabilized sludge is produced, which eventually leads to a reduction of cost to dispose sludge.
4. Consumption of low energy is common to anaerobic systems. Anaerobic pretreatment serves as equalization basin, where diurnal variations of oxygen demand could be reduced, leading to aeration and energy requirements.
5. In presence of volatile organics in wastewater, the volatile organics compounds are degraded anaerobically, reducing the tendencies of volatilization during aerobic phase of treatment.

Several authors (Table 2.8) have reported use of various anaerobic-aerobic systems to treat FBI wastewaters, with influent COD ranging from 345 to 7,900 mg/L. Based on available data, anaerobic-aerobic systems can achieve COD removal efficiency ranging from 80 to 98.7 %, with OLR ranging between 1.6 to 7 kg COD/m^3 d. However, inspite of the potentials of anaerobic-aerobic systems, some drawbacks include the fact that nitrate generated in aerobic zones due to oxidation of organic matter will be untreated. Thus, released into the environment with possible consequences highlighted in Section 2.3. Thus, prospects to achieve stringent effluent requirements of TN requires anoxic condition to reduce nitrate levels safe for discharge into receiving water bodies

Table 2.8: Integrated anaerobic and aerobic systems to treat food industry wastewater

S/no	Bioreactor type	Type of wastewater	Influent COD (mg/L)	OLR (kg COD/m^3 d)	Total COD removal (%)	Anaerobic COD removal (%)	Aerobic COD removal (%)	Total HRT (h)	Anaerobic HRT (h)	Aerobic HRT (h)	Ref.
1.	SAA bioreactor	Diluted landfill leachate	1000-3300	-	84	-	-	3-11	-	-	[243]
2.	RAAIB bioreactor	Sewage	345	-	84	-	-	1.2-15.5			[244]
3.	AAIBR	Potato starch ww	1100-4500	-	88.4-98.7	87	-	1.2-15.5	-	-	[245]
4.	JBILAFB	Food processing	960-7900	1.6-5.6	80	-	-	24			[224]
5.	UASB+AS	Olive mill ww	1800-4400	3-7	95-96	70-90	>60	28.3	14.7	13.6	[246]

Bioreactor type- SAA: Simultaneous aerobic and anaerobic bioreactors, RAAIB: Radial anaerobic/aerobic immobilized biomass, AAIBR: Anaerobic aerobic integrative baffled reactor, JBILAFB: Full-scale jet biogas internal loop anaerobic fluidized bed, UMAR: upflow multistage anaerobic reactor; UASB: upflow anaerobic sludge blanket, AS: activated sludge, ww: wastewater.

2.12.1.2 Biological nitrogen removal perfomance with various configurations

Lee et. al. [247] proposed a modified Ludzack-Ettinger (MLE) type membrane bioreactor as a method for treating food disposer wastewater. The process was shown to be effective with a high COD/nitrogen ratio of 20. Removal of both organic matter and total nitrogen can be achieved according to some BNR configurations shown in Table 2.9.

Table 2.9: Performance of some BNR configuration processes

Configuration	HRT (h)	R Q_{IR}/Q_{IN}	Total SRT (d)	Anoxic/ Aerobic (v/v) ratio	Removal (%)	Ref.
UBR-A Process UBR, Aerobic, ST	7-9	0.333	14-50	0.57-0.73	COD: 90-94% TN: 59-76%	[248]
IVMBR	6.67	4	30	1:1	COD: 95% TN: 76%	[212]
UASB-A/O	1.75	4	82.6	1:3	COD: 95.6% NH_4-N: 99.5%	[249]
UASB-AS	23-39	1-3	51-75	0.63	COD: 97%, TN: 77%	[250]
MLE	6	4	12	1	NH_4-N: 17-98%	[251]
A^2/O	96-336	1-4	-	1	COD: 96% N: 50%	[252]

UBR: Upflow bioreactor, IVMBR: Integrated memebrane bioreactor, UASB-A/O: Upflow anaerobic sludge blanket anoxic/aerobic, UASB-AS: Upflow anaerobic sludge blanket activated sludge, MLE: Modified Ludzak Ettinger process, A^2/O: Anaerobic/Anoxic/Aerobic

2.13 Influence hydraulic retention time and internal recycle on denitrification

Influence of HRT and recycle (R) rate are important parameters to influence high degree of nitrogen removal in wastewater treatment [193, 253]. HRT is expressed as the ratio of total reactor volume to influent flow rate and represents the average time the liquid resides within the reactor. The total HRT of a system is determined by the influent flow rate, whereas the actual HRT in the reactor is controlled by both the influent flow rate and R streams. A lower HRT could result in higher loading and influent flow rates, which increases the hydraulic and substrate loading rates, and affect removal performance [254]. Kim et al. [253] studied pre-deniyrification of industrial

wastewater and found that removal efficiency significantly decreases with a decrease in the HRT. Conversely, a high HRT increases the solubilisation of organic matter in both colloidal and particulate form and supports nitrification [255]. In spite of this, a high HRT may prove uneconomical, necessitating the need for establishing a balance between cost and removal efficiency [256].

The R ratio determines how much nitrate formed during the nitrificatin process is transfered to anoxic region for denitrification. In the case of strong nitrogenous wastewaters there are positive effects for reduction of toxic effects due to high ammonia concentration as a result of dilution due to combined flow. In BNR process, the R ratio are normally between 1 and 4 [115]. For a typical MLE type configuration, IR flow is more sensitive as the non recycled nitrates will escape with the effluent. If the denitrification performance is not limited by the availability of substrate and microbial kinetics, then effect of IR on system denitrification potential can be expressed according to Equation 2.16 [257].

$$P_{D-IR}(g/N/d) = \frac{R}{1+R} TN_{INF} Q_{IN} \qquad (2.16)$$

Where $P_{D\text{-}IR}$ is the denitrification potential with respect to internal recycle, R is the internal recycle ratio ($R = Q_{IR}/Q_{IN}$), $TN_{INF}$is the total nitrogen concentration and Q_{IN} is the influent wastewater flow rate. Using the expression from Equation 2.16, effect of increasing R on the denitrification efficiency (%) in a pre denitrification system like MLE is shown in Figure 2.5.

There will be increase in denitrification potential until an R of 3 to 4, beyond which denitrification is not improved considerably. This condition could warrant recycling more liquour with DO content. Oxygen as an electron donor is more favored by denitrifiers (facultative heterotrophs) over nitrate. 8 % more percent of energy is supplied by oxygen and 50 % more cellular growth per unit of oxygen compared to same amount of nitrate utilized [167]. Hence, recycling mixed liquor with high DO content can inhibit denitrification performance [257].

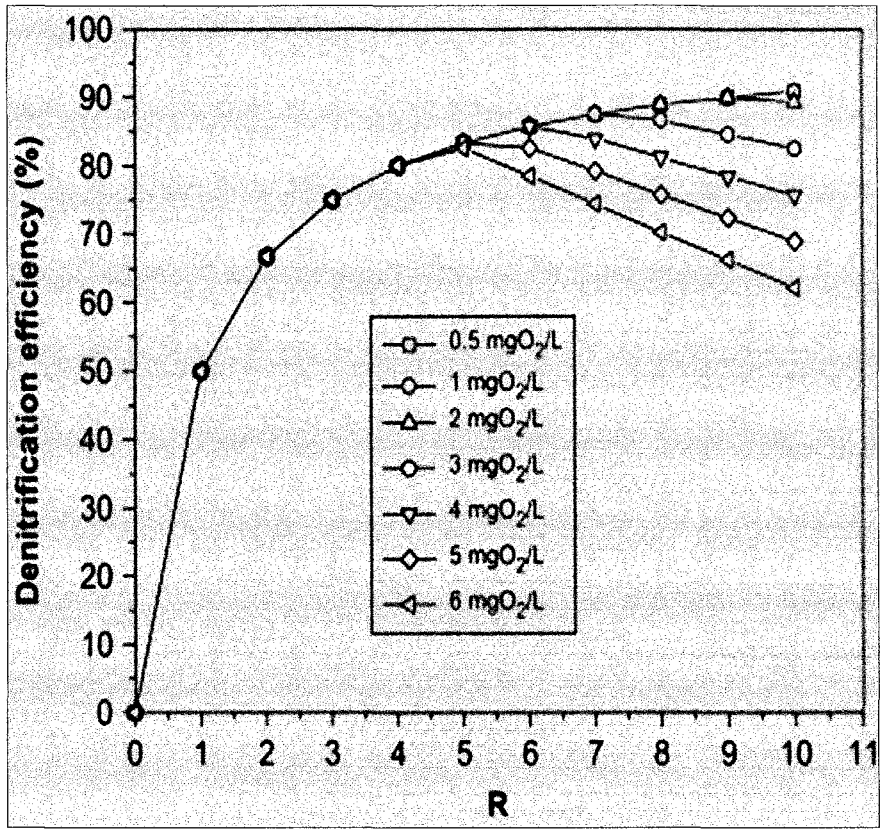


Figure 2.5: Graphical illustration of denitrification efficiency with R [257]

2.14 Influence of solids retention time

The nitrifiers (AOA and NOB) in the BNR are inherently slow growers, therefore, SRT enhances their development [258]. Heterotrophic bacteria grows faster than nitrifiers. Less energy is acquired from ammonia and nitrite oxidation, thus, growth rate of nitrifiers is 10-20 times slower than heterotrophic bateria, consequently higher SRT is requisite in order to maintain sizeable consortia [167]. Variety of new and diverse bacteria population have been reported at longer SRT for ASP with observed low sludge formation [259]. Increased nitrogen removal was observed with a system having anoxic/aerobic configuration operating at a longer SRT, where lower SRT was reported to be more vulnerable for biomass washout [260]. Although, keeping higher in the system could lead to biomass accumulation, thereby with tendencies of rising inert materials fraction having less active microorganisms and low substrate [261].

2.15 Monod's model for microbial growth and substrate utilization

The production of sludge is a specific feature of the activated sludge process. The utilization of the substrate by the microorganisms result in the growth of new biomass [262]. The knowledge of biokinetics in wastewater treatment is essential for appropriate design and optimization of operational conditions [263, 264] [265].

The Monod's equation [266] is generally accepted by the International Association on Water Pollution Research and Control (IAWPRC) task group as the fundamental basis for development of the Activated Sludge Models (ASM) [267], and most frequently used model to describe the interaction between growth of microorganisms and utilization of the growth limiting substrate [84, 85, 145, 268]. Monod equation is a kinetic model which describes microbial growth as a functional relationship between the specific growth rate and essential substrate concentration [269].

There are existing additional models which describe microbial growth and substrate degradation kinetics, but the Monod's formulation has the benefit of its simplicity, accurate and comparatively expressive [270]. The graphic representation of Monod's equation is shown in Figure 2.6.

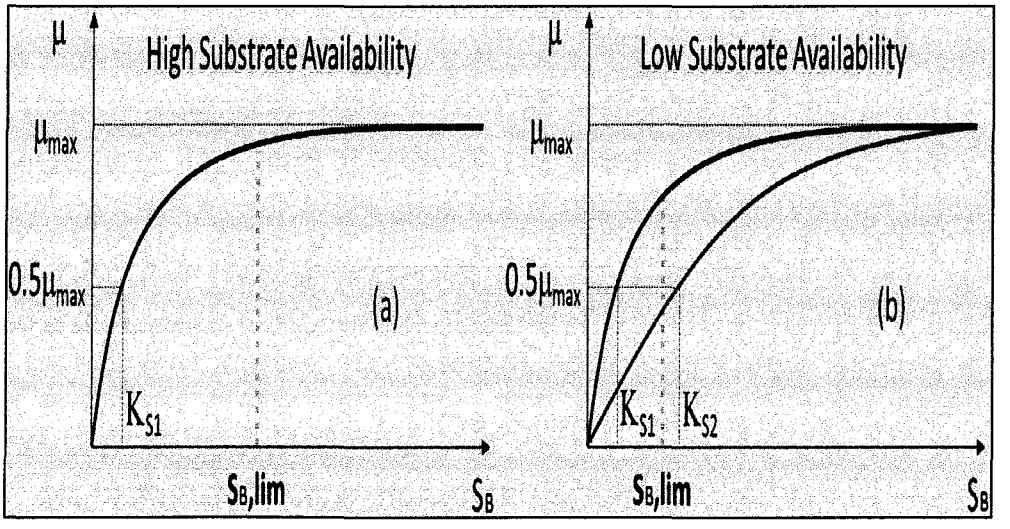


Figure 2.6: Illustration of Monod's parameters limiting growth kinetics at different substrate availabilities [271]

Monod model's distinct formulation allows changing from zero-order growth kinetics at high substrate concentration to first-order growth kinetics at low substrate concentrations, so as to imitate actual microbial behavior [271]. Figure 2.6a illustrates the high availability of readily biodegradable substrate (S_B), growth kinetics are independent of substrate concentration and instead are determined by the maximum specific growth rate (μ_{max}). In contrast, at low substrate availabilities, growth kinetics become substrate limited and the so-called "half-saturation constant" or "affinity constant" (K_S), substrate concentration at which the growth rate corresponds to half the (μ_{max}) is the main parameter influencing growth rate (Figure 2.6b). In low substrate availability conditions, microbial competition for substrate becomes a relevant phenomenon. It is common process understanding that the organisms with the highest affinity towards the substrate with lower K_S will outcompete the other ones present in the culture. This understanding can be explained using the Monod equation in Figure 2.6b. The organism with the lower K_S (K_{S1}) presents higher growth rates at low substrate availabilities than the organism with a higher K_S value (K_{S2}), given that μ_{max} is the same. From the foregoing, it follows that at low substrate availabilities, process performance according to the model will be determined to a large extent by the value of the "half-saturation constants". Previously, the operation of wastewater treatment processes has been closer to the situation shown in Figure 2.6a than to the one shown in Figure 2.6b, especially in systems with low solids retention times, with specific maximum growth rates determining the rates and extent of contaminant removal [272]. Numerous industrial wastewaters, including food processing wastes, regularly have colloidal and particulate organics that undergo hydrolysis prior to biodegradation [273]. The kinetic modeling of hydrolysis in wastewater treatment has been reviewed [274], with the opinion that most widely used kinetic model was first order with respect to the particulate substrate [275, 276].

2.15.1.1 Some past studies to determine biokinetic coefficients using Monod's model

Some reported kinetic studies conducted on food wastewater is discussed here. El-Kamah et al. [277] made attempts to determine the biokinetic constants for heterotrophic bacteria in an SBR operated system with three different organic loading

rates (OLRs): 2, 1.7 and 1.1 kg COD/m^3 d. The results of continuous long-term operation showed that by decreasing OLR from 2 to 1.7 kg COD/m^3 d, there was an increase observed in removal efficiency from 95.5-99.3% for COD, from 95.3-98.1% for BOD and from 87-97.7% for TSS. Whereas, with further reduction in the OLR to 1.1 kg COD/m^3 d, there was no substantial effect on COD or BOD removal. Additionally, remaining total nitrogen concentration decreased by decreasing the OLR. Although, increasing the OLR brought slight drawback on the removal of total phosphorous. For the determination of the biokinetics coefficients, the experimental data indicated that the substrate utilization kinetics are consistent with Monod's kinetics model approximately. The maximum specific growth rate (μ_{max}), half velocity coefficient (K_s), growth yield coefficient (Y) and decay coefficient (K_d) were determined as 2.94 d^{-1}, 15.22 mg/L, 0.2384 g VSS/g COD and 0.2019 h^{-1}, respectively.

Durai et al. [278] conducted a study on aerobic digestion from tannery wastewater industry using mixed culture sourced from effluent of the treatment plant of the tannery wastewater. The wastewater effluent COD concentration was found to be 1,560 mg/L which was used as the substrate into the aerobic reactor. Kinetics studies were carried out using Monod model, First order, Diffusion model and Singh model. The results obtained for the kinetic study found that the Monod model fits well for the degradation of tannery wastewater using mixed microbial consortium.

Lateef et al. [279] carried out research to evaluate the performance of laboratory scale biological treatment process for a diary industry wastewater and to determine bio-kinetic parameters for an activated sludge process. The experimental set up consist of an aeration tank and a clarifier for settling the biomass which was operated continously for three months. Parameters such as HRT were varied from 2-12 d. The influent and effluent BOD as well as the MLSS of the aeration tank were determined at various detention time to generate data for the kinetic co-efficients. The kinetic co-efficients k (maximum substrate utilization rate), K_s (half velocity constant), Y (cell yield co-efficient) and K_d (decay co-efficient) were obtained as 4.46 d^{-1}, 534 mg/L, 0.714, and 0.038 d^{-1}, respectively.

Batch scale ASP kinetic studies were carried out by Nakhla et al. [273] to treat pet food wastewater categorised by oil and grease concentrations. The concentration levels were up to 21,500 mg/L for COD, and BOD concentrations of 75,000 and 60,000 mg/L, respectively as well as effluent from the batch dissolved air flotation (DAF) system. The results of the kinetics studies showed that Monod model kinetic constants for the raw wastewater i.e., k, K_S, Y, *and* K_H varied from 1–1.3 gCOD/gVSS d, 5580–5600 mgCOD/L, 0.08–0.85 mgVSS/mgCOD, and 0.21–0.66 d^{-1}, respectively.

Mardani et al. [280] undertook a research study to determine the bio kinetic coefficients and performance to determine efficiency of three activated sludge process plants. These plant comprise conventional, extended aeration (EA), and contact stabilization. Research was conducted for period of six months as pilot-scale at Isfahan south municipal WWTP. Two regimes were changed for the MLSS concentration in aeration chamber.The operation was conducted in five stages for each MLSS concentration, flow rate and solids retention time (SRT). Results for investigation presented kinetics co-efficients as: yield constant (Y), decay coefficient (K_d), maximum specific growth rate (μ_{max}) and saturation constant (Ks) for the conventional ASP observed in range of 0.48-0.8 mg VSS/mg sCOD, 0.0189-0.026 d^{-1}, 0.95-0.98 d^{-1} and 52-71 mg sCOD/L. Corresponding values for the EA kinetic co-efficients were obtained as; 0.6174-1.2512 mg VSS/mg sCOD, 0.0198-0.0309 d^{-1}, 1.96-3.17 d^{-1} and 311.7-508 mg sCOD/L, and for contact stabilization 0.6322-0.713 mgVSS/mg sCOD, 0.0172-0.0387 d^{-1}, 0.23- 0.42 d^{-1} and 13.8-50.8 mg sCOD/L, respectively. The kinetic co-efficient values for conventional and contact stabilization processes were similar to previous findings reported in literature. But, values of EA process for K_s and Y with MLSS of 5,000 mg/L were not within normal range reported. Sensitivity analysis for variations in bio-kinetic coefficients express relationship of K_d and Ks as direct with effluent substrate concentration. However, μ_{max} was inversely related to the concentration of effluent substrate. Additionally, irrespective of substrate source and concentration of aeration chamber MLSS, effluent substrate concentration was observed as more sensitive to μ_{max} than K_d and Ks. The results also showed performance for COD removal efficiency in conventional system ranges between 83 to 92.5%, EA between 88 to 93.8%, and contact stabilization system between 77 to 92 %.

2.16 Chapter summary and further studies

Various systems to treat beverage wastewater have been studied, which include anaerobic, anoxic and aerobic processes. Though, some significant demerits exist for conventional systems such as use of large space for installation, odorous smell and production of excess volume of sludge. Sludge disposal in landfills, land application or incineration, or as post treatment alternative could attract substantial efforts to manage. Hence, one of the feasible strategies to tackle surplus sludge disposal is to reduce its production at operational stage. Outcome from this review indicated that anaerobic/aerobic bioreactors can be effective in treatment of beverage wastewater, the process however, is limited to provide adequate removal of TN because oxic environment alone as the case of ASP treatment basin remains unfavorable for denitrification [281]. Therefore, denitrification alternatives must prevail to effectively reduce nitrates in a separate reaction chamber [282]. Release of these nitrates into water bodies has been linked to endogenous formation of nitroso compounds (such as nitromines and nitrosomides), which have been reported as potential carcinogens [63, 283]. Overall observation has shown that COD removal efficiency is generally lower when organic loading rates are high [26, 253]. Although, suspended growth bioreactors are operated with lower organic loading rates, whereas, attached growth bioreactors could relatively be operated at higher organic loading rates to treat wastewater. It is recommended that aerobic treatment process could proceed with COD concentration lower than 1,500 mg/L, and anaerobic treatments with much greater COD concentration values of up to 50,000 mg/L, which can be further reduced to manageable concentration from successive treatment [85, 284].

Based on identified gaps established in literature review, major operational conditions controlling loading rates, hydraulic retention time, solids retention time are indicated with significant effect on biodegradation process of organic matter, nitrification and denitrication performance. Considering these dynamics, there is need to study underlying control parameters within integrated suspended growth bioreactor operation. This process combines treatment sequence with mediums of anoxic, aerobic and aerobic digestion of sludge, in a view to accomplish stable and optimum treatment to remove carbon, nitrification, denitrification and simultaneous reduction of sludge.

CHAPTER 3

METHODOLOGY

3.1 Chapter overview

This chapter describes the materials and methods used in this research. Consequently, in this section details will be given explaining the site description of the research collaboration industry, characteristics of the wastewater used for the pilot study, experimental design detailing the experimental phases, an analytical procedure for the physiochemical parameters, sampling plan and techniques for performance evaluation are presented in this chapter.

The research was conducted in three phases. These phases include the following:

i. Phase I: Concept development, design, and fabrication:

Bioreactor was designed to have anoxic chamber receiving influent wastewater from beverage industry, followed by aerobic chamber, and settles the biomass in clarifier. Aerobic digestion chamber was designed as a stand-alone chamber to degrade excess activated sludge. Bioreactor was fabricated by Solution Engineering Sdn. Bhd. in Malaysia.

ii. Phase II: Operation with aerobic digestion:

This phase was the start-up, operation and simultaneous sludge digestion using i-SGBR system, and it comprises of four steps;

a. Phase II-A as start up, biomass build, acclimatization and steady state operation with influent flow rate of 45 L/d, IR of 3 and , then

b. Phase II-B was operation with influent flow rate of 45 L/d, IR of 6

c. Phase II-C was operation with 70 L/d, IR of 6 and 3, and

d. Phase II-D was operation with 100 L/d, and IR of 6.

iii. Phase III: Bio-kinetic study with aerobic digestion

This phase comprises of five steps operation to generate data for the bio-kinetic parameters. The operation to determine the bio-kinetic coefficients for carbon oxidation was conducted using Modified Monod's model with the following consideration;

a. Phase III-A was operated with an influent flow rate of 100 L/d, SRT of 40 days and AER-C HR of 30 hours.

b. Phase III-B was operated with influent flow rate of 110 L/d, SRT of 35 days and AER-C HRT of 27.3 hours,

c. Phase III-C was operated with influent flow rate of 120 L/d, SRT of 30 days and AER-C HRT of 25 hours,

d. Phase III-D was operated with influent flow rate of 135 L/d, SRT of 25 days and AER-C HRT of 22.2 hours, and

e. Phase III-E was operated with an influent flow rate of 150 L/d, SRT of 20 days and AER-C HRT of 20 hours.

From the foregoing, experimental phases are expressed and segmented by the methodology framework according to Figure 3.1. Operation of aerobic digester was carried out based on batch feeding at HRT of 24 hours and SRT of 10 days , where the aerobic digester was fed and decanted daily with a sludge volume of 7.5 L/d from the clarifier underflow. Wasting of sludge was considered from the waste-line of waste activated sludge at clarifier underflow.

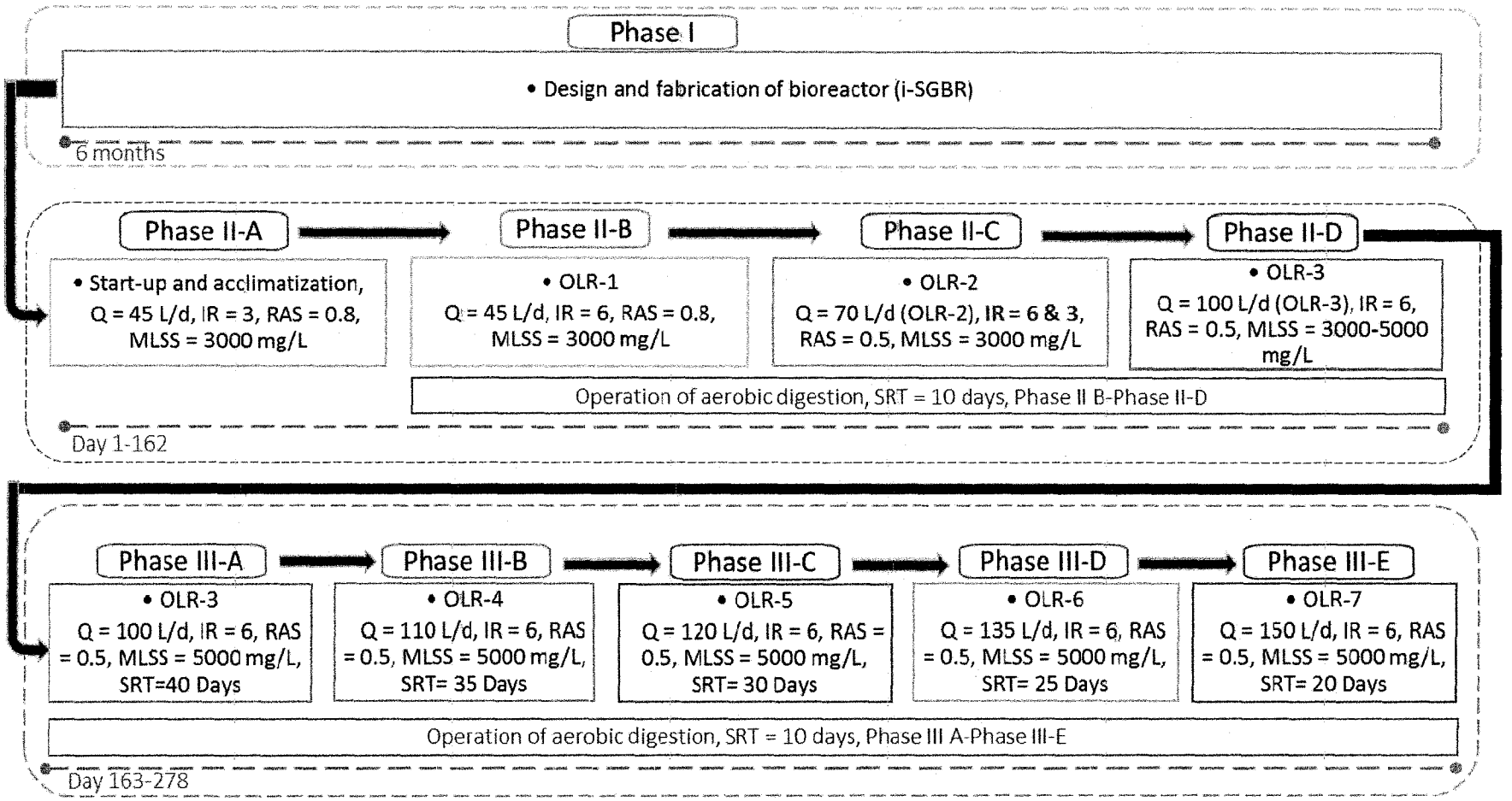


Figure 3.1: Flow chart for the methodology framework

3.2 Site description and existing treatment system

The wastewater for the site originates from FBI, which is a franchised company producing can products, predominantly brands of Milo and Nescafe for a conglomerate, Nestle. Daily activity routine include 18 hours production and 6 hours for cleaning. As gathered from the industry's inventory, average volume of the wastewater produced daily ranges between 40–60 m^3/d.

The company's current wastewater treatment system is extended aeration activated sludge system. Figure 3.2 illustrates flow chart of the existing treatment process. The main goal was to reduce level of organic matter and treatment of ammonia from the effluent wastewater produced due to production activities. This was in view to conform with local legislation prior to its discharge to the natural environment.

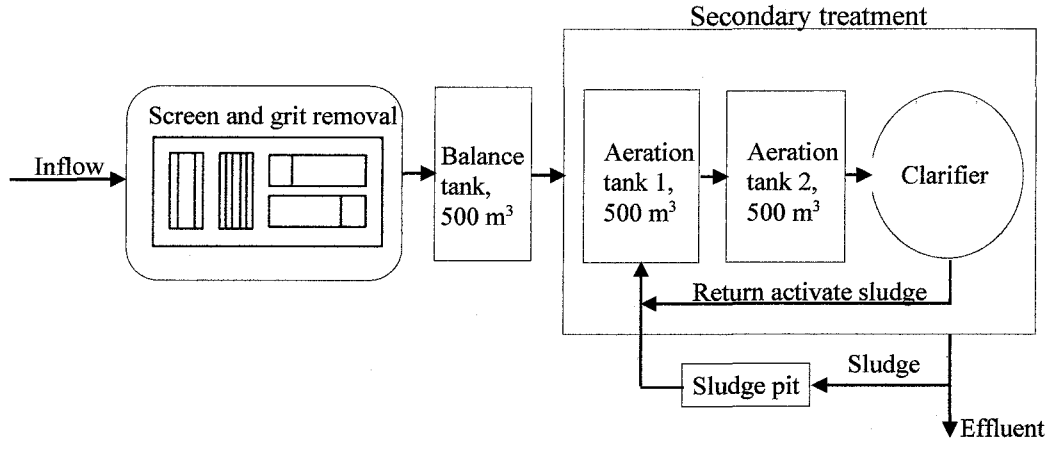


Figure 3.2: Current activated sludge wastewater treatment system

The treatment method is a traditional practice adopting conventional aerobic wastewater treatment system. This comprises physical treatment (preliminary pre-treatment) which was mechanically operated to screen and remove grit, and removal of large particles from the wastewater that were separately collected and disposed of. The wastewater was then channelled and stored in balance tank reservoir with a capacity of 500 m^3. The next process was the secondary treatment or biological treatment, where there were two extended aeration tanks (tank 1 and tank 2), each with

capacity of 500 m^3 that operated in series. The objective of the secondary biological treatment is to oxidize the organic matter and remove the non-settleable colloidal solids. The clarifier was to settle the biomass following secondary treatment, and settled biomass was returned to the aeration tank 1 with supernatant discharged as effluent. The RAS was frequently returned to the aeration tank 1 to maintain the biomass concentration.

3.3 Source of influent wastewater for the pilot study

To operate i-SGBR system, initial stages entail prerequisite information to characterize the wastewater. Influent features of wastewater for the study were analyzed. The parameters such as COD, sCOD, BOD_5, TSS, TP, ammonia-nitrogen, nitrate-nitrogen, total nitrogen, TKN, and pH were determined.

Required quantity of wastewater was pumped from the industry's WWTP balance tank (500 m^3) to the i-SGBR system feed tank as illustrated in Figure 3.3. The quantity pumped during phase II was 45 L, 70 L and 100 L, and during phase III, 100, 110, 120, 135, and 150 L were pumped during various steps of the experiment. The pumping was automated by a submersible pump with a capacity of supplying 50 L/m fitted with a timer.

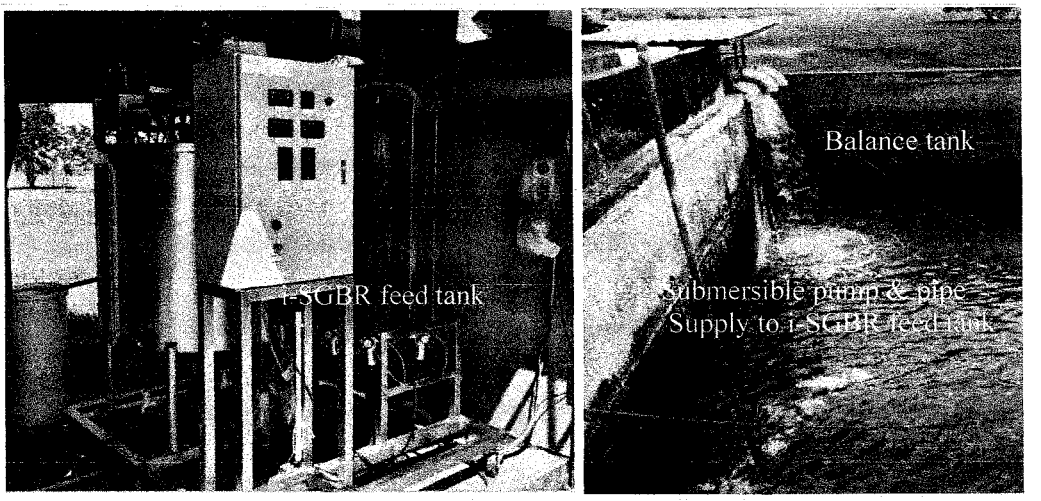


Figure 3.3: Illustration of the wastewater feed transfer to pilot i-SGBR

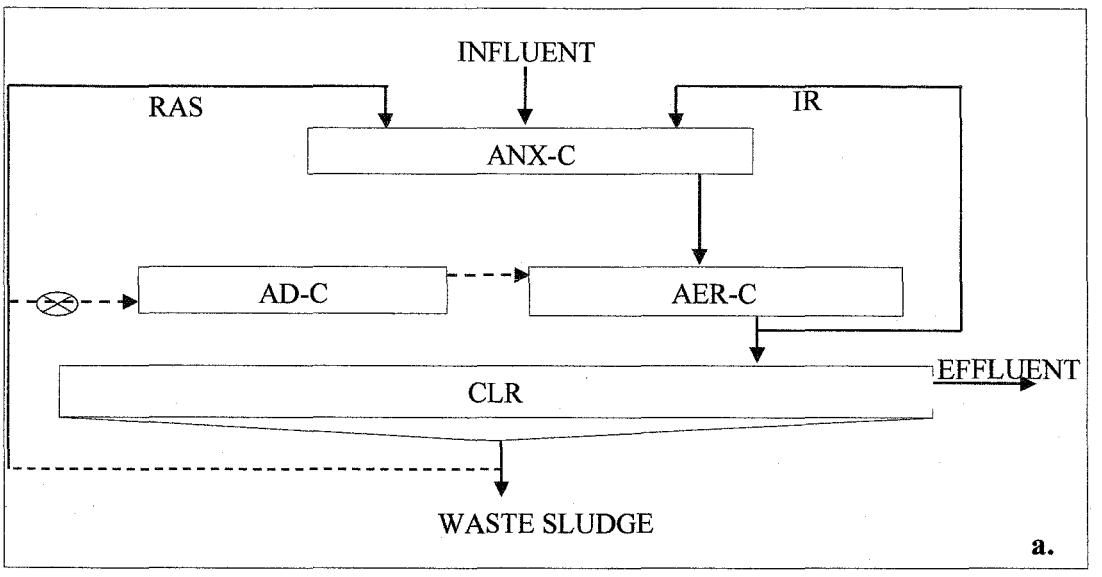
3.4 Experimental scheme for the operational phases

In this section, operation and control parameters, experimental design and performance of the system for each phase is discussed in order to achieve the specific objectives. Section 3.4.1 details the concept, design, and fabrication, Section 3.4.2 explains the start-up and operation in phase II, and Section 3.4.3 explains the determination of bio-kinetic coefficients for carbon oxidation for phase III operation.

Activity in each phase was operated until its steady state conditions were reached, then only the next successive activities were carried out. Steady operation of the process was assumed to be reach when change in concentration with time in effluent was observed. In earlier researches, steady state was presumed at obtaining values of standard deviation for the mean efficiencies of COD removal, nitrification and denitrification when they were less than 10% in variability [285, 286]. Generally, biological treatment methods are certainly variable as a result of changes due to influent loadings, external uncertainties and internal dispositions in the microbial state [287, 288].

3.4.1 Phase I Experimental scheme: Bioreactor concept, design, and fabrication

In this research, an innovative integrated suspended growth bioreactor system (i-SGBR) was designed, developed and operated as a pilot plant. The compact system was operated to evaluate the effectiveness for removal of organic matter (COD and BOD_5), nitrogen, suspended solids, degradation of excess sludge, and the biomass settling in CLR. The conventional system described in Figure 3.2 has the treatment systems segregated. Thus, the concept of i-SGBR has all the treatment chambers integrated within a single system. The treatment chambers of i-SGBR were constructed and combined together, through ascending the treatment units vertically upward as alternative to disperse radially. Treatment chambers utilize common wall area separation in between the chambers. Performance of i-SGBR was assessed through the degradation of the organic matter, elimination of total nitrogen, and simultaneous reduction of sludge in the system. The conceptual design of i-SGBR system is shown in Figure 3.4 a. and b.



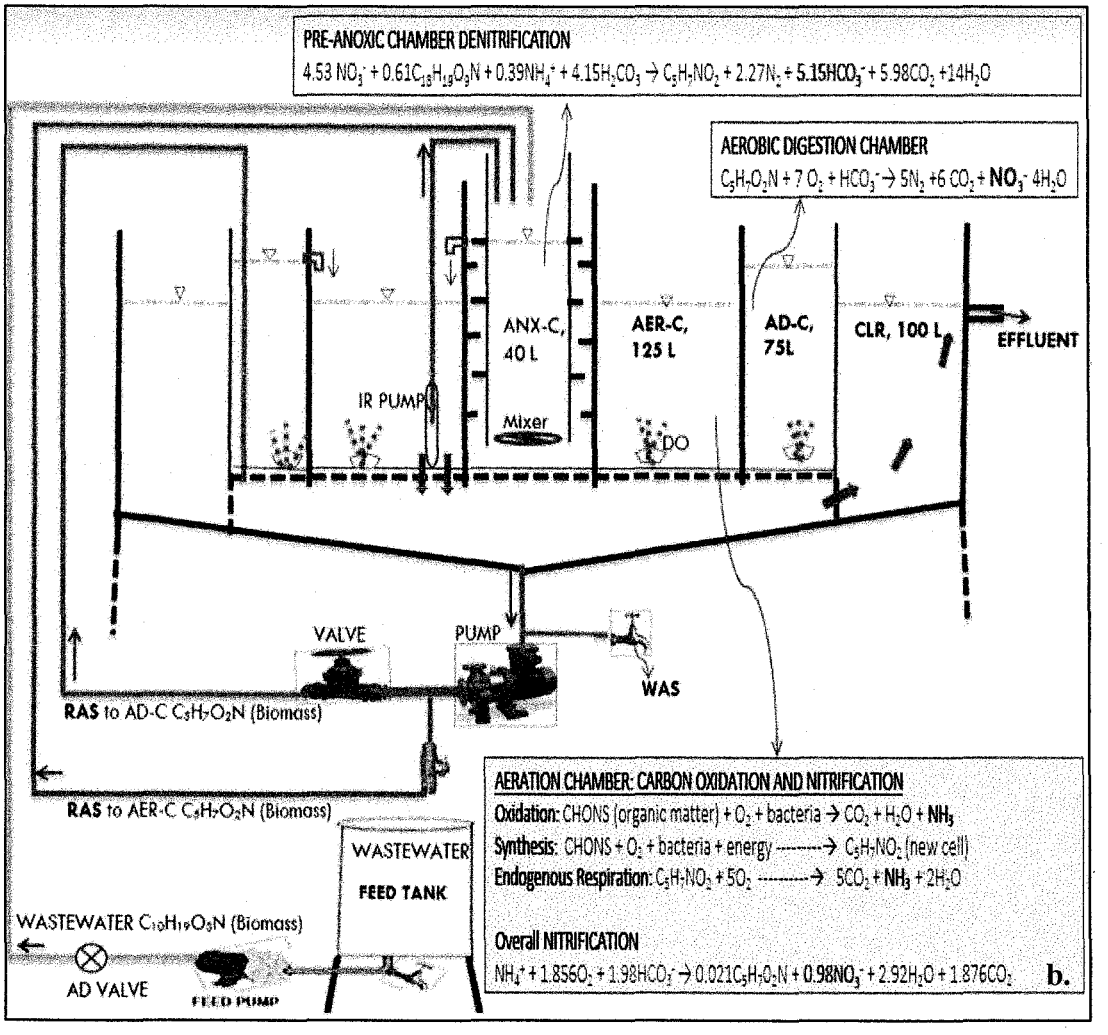


Figure 3.4: The conceptual design and biochemistry of i-SGBR system

Wastewater in i-SGBR feed tank (150L) was supplied into ANX-C. ANX-C utilizes wastewater as carbon source electron donor for denitrification. E-ANX-C discharges into AER-C. Nitrates produced in AER-C and AD-C is pumped to ANX-C through internal recycle (IR) pump, terminal electron acceptor is nitrate [193].

Stoichiometry for overall denitrification process was described in Equation 2.10 in Section 2.7.3.2. Nevertheless, the ANX-C has the advantage to recover alkalinity and DO from the denitrification process. Three distinct processes occurring in the AER-C are; oxidation, synthesis and endogenous respiration of organic matter [61, 84].

The carbon oxidation process, synthesis, and endogenous stoichiometry are described in Equation 2.1 and Equation 2.2 in Section 2.5.1. Nitrification process produces ammonia nitrogen, which ultimately is oxidized to nitrate, and subsequently, nitrate is reduced in the ANX-C to nitrogen gas [289]. Stoichiometry of nitrification process is described in Equation 2.6 in Section 2.7.2.2.

Oxidation process requires DO and results in the destruction of alkalinity, thus with tendencies of lowering the pH of the aerobic system. Recycle of thickened RAS in CLR was done through the intermittent recharge of biomass, so as to keep concentration of biomass in the aeration chamber [290].

AD-C was operated as a separate unit, periodically supplied with sludge through underflow and held until required HRT. The stoichiometry of the AD process is described by Equation 2.12 and Equation 2.13 The i-SGBR AD-C resembles the conventional AD process, where the addition of sludge to an aerated basin was done daily, and the SRT is greater than the HRT [85]. In the intermittent process, the solids are added and removed from the AD periodically, usually once per day. Using the formula $C_5H_7NO_2$ to symbolize mass of a typical cell of microorganism [112, 208], the stoichiometry for the aerobic digestion can be expressed according to Equation 2.14 and Equation 2.15 in Section 2.9.1.

Equation 2.14 represents the system inhibiting nitrification, where nitrogen is released in the form of ammonium. While, for a system in which nitrification occurs, is expressed by Equation 2.15. The aerobic digestion process is similar to extended aeration process with common endogenous metabolism environments [85]. The degradation manner continue from hydrolysis of biodegradable organic matter, and conversion to soluble organic matter, with release of nutrients. The next sequence is transformation of biodegradable soluble organic matter to carbon dioxide, water, and active biomass with help of heterotrophic bacteria. Successively, active biomass proceeds through decay process to produce carbon dioxide and water, and extra inactive biomass as cell debris [200]. Non-biodegradable part of particulate matter from influent will be unchanged, and remain part of digested solids. Aerobic digestion results in the degradation of MLVSS and MLSS [85, 203, 204].

System SRT control was achieved by MLSS concentration in AER-C from sludge wasting rate. Sludge wasting was necessary to get rid of a non-biodegradable portion of inert matter accumulation in the system [268, 291]. The final settler serves as a separator to settle the biomass and supernatant wastewater was discharged.

The i-SGBR system was then designed and operated as a continuous flow suspended growth system, resembling upsteam denitrification and downstream extended aeration process, and addition of aerobic digestion system. Design was according to the outlined process explained elsewhere [84, 113]. The process design calculations for combined removal of organic matter and nitrification and denitrification were prepared based on adapted bio-kinetics parameters [84]. The design of i-SGBR was based on the temperature of 28 °C, where temperature correction was done according to the Arrhenius Equation 2.7 in Section 2.7.2.1. The values of bio-kinetic coefficients are provided in Table 3.1.

The designed volumes were calculated and obtained for ANX-C, AER-C, AD-C, and CLR. A concentric geometry was considered in the design of i-SGBR system, where the individual chambers for each treatment unit were concentrically placed radially from the center. The innermost concentric chamber is ANX-C, then followed by the AER-C, next to AER-C is the AD-C (stand alone process), and finally clarifier.

An overview of i-SGBR system is illustrated in Figures 3.5 through 3.6 showing the top view, bio-reaction chambers (ANX-C, AER-C, AD-C, and CLR), rear view and the approach view.

Table 3.1: Values adopted for the kinetic coefficient for design

Description	Coefficient	Value	
Biomass yield co-efficient for heterotrophic bacteria	Y	0.4	gVSS/g bCOD
Half – velocity constant	K_S	20	g/m^3
Fraction of biomass remaining as cell debris	f_d	0.15	-
Half-saturation coefficient for DO	K_O	0.5	g/m^3
Biomass yield co-efficient for nitrifying bacteria	Y_n	0.12	gVSS/gNH_4^+-N
Maximum specific growth rate of nitrifying bacteria	$\mu_{n,m}$	1.29	g/g.d
Half-velocity constant for nitrification	K_n	1.12	g/m^3
Endogenous decay coefficient for nitrifying organism	K_{dn}	0.11	g/g.d
Specific growth rate of nitrifying bacteria, g new cells/g cells.d	μ_n	0.21	g/g.d
Endogenous decay coefficient	K_d	0.16	g/g.d
Maximum specific growth rate of heterotrophic bacteria	$\mu_{m,T}$	10.3	g/g.d
Temperature, t		28	°C

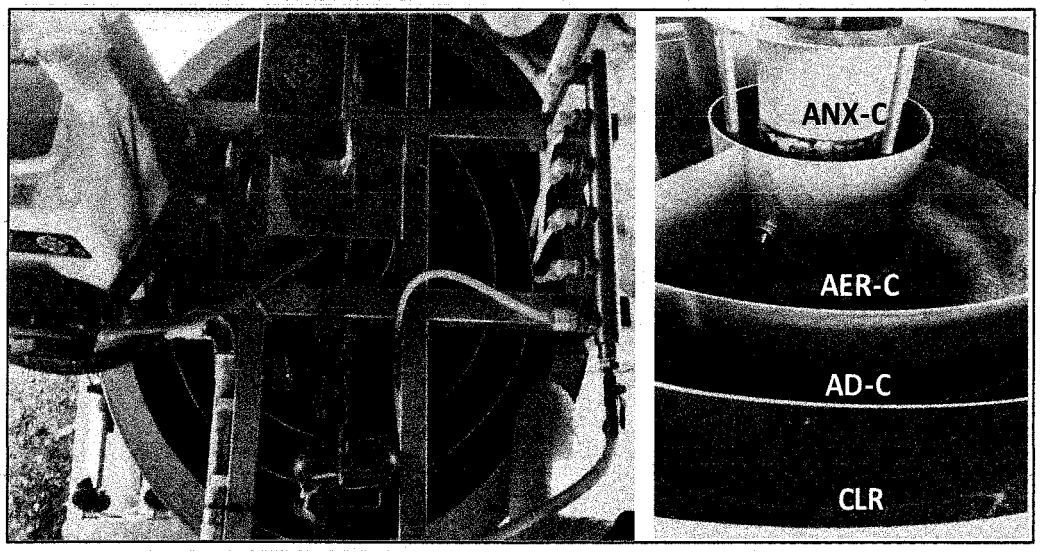


Figure 3.5: Top view of i-SGBR treatment chambers

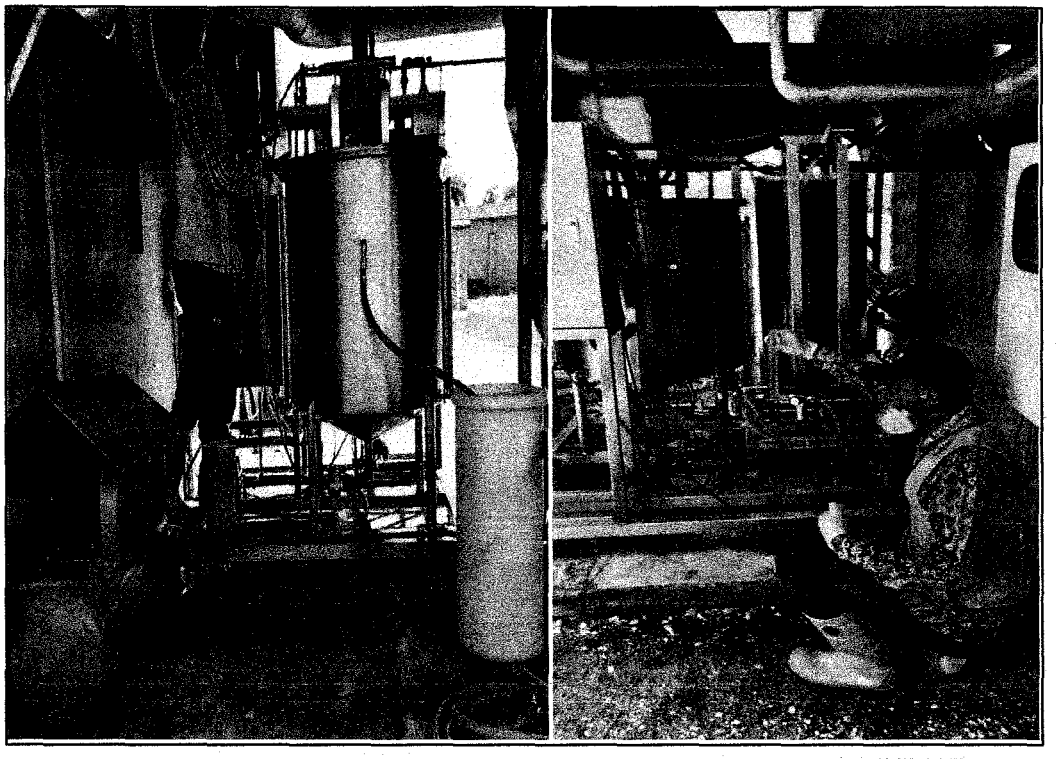


Figure 3.6: i-SGBR rear view & approach views

Modified Ludzack-Ettinger (MLE) process was adapted for the treatment configuration [84, 114, 178, 292]. The MLE process is one of the most widely used processes to remove nitrogen from wastewater, although most plants using the MLE process are operated at constant DO in the aerobic zone and constant internal recycle flow (IRQ) to the anoxic zone [293]. Thus, the i-SGBR comprises an upstream anoxic zone followed by the aerobic zone where nitrification occurs in the aerobic zone and nitrate is then recycled back to the anoxic zone for denitrification [114].

3.4.2 Phase II Experimental scheme for the operation

The start-up and operation of the i-SGBR for the experiment in phase II are described here. Phase II was operated between day 1-162 in suspended growth mode treating FBI wastewater. The objective was to monitor and evaluate the performance of i-SGBR on effect HRT and IR with flow rates of 45, 70 and 100 L/d and operate simultaneous sludge degradation. The volumes of ANX-C, AER-C, AD-C and CLR were fixed throughout the duration of the experiment.

3.4.2.1 Phase II operation and control parameters

System operation and control parameter such as MLSS, MLVSS, SVI, pH, temperature, DO and food to micro-organisms (F/M) ratio were monitored. i-SGBR system was operated for 40 days SRT and AD-C for 10 days SRT. Microbial parameters such as heterotrophic plate count (HPC) and determination of microfauna such as protozoa and metazoa was carried out from the sludge samples in the aeration chamber.

Solids balance was performed for both MLSS and MLVSS within the AD-C system boundary according to the procedure defined in previous research [84, 294, 295]. The determination of the actual volatile solids reduction efficiency in an operating aerobic digester should be based on the mass flow rates of VSS entering and leaving it. Nevertheless, some sources [204] use the percent VSS content of the feed and effluent solids to make this calculation, it susceptible to inaccuracy due to the procedure does not consider changes in the fixed suspended solids and accumulation in the aerobic digester system [85].

A comparison was made between the degraded mass from the effluent aerobic digester (EAD) and wasting rate (Q_W. X_r) from CLR underflow, in order to maintain the desired SRT in the i-SGBR AER-C. The aerobic digester efficiency and the operational SRT of the AER-C are key variables to determine the wasting rate. Equation 3.1 is the general concept for the expression of mass balance [84], and Figure 3.7 presents schematic diagram of the aerobic digestion mass balance principle [294].

$$\textit{Overall change in mass} = \sum IAD - \sum EAD \pm \textit{Net change within AD-C system} \quad (3.1)$$

This process includes the mass entering the aerobic digester (IAD), the accumulation in the aerobic digester [$\Delta(C_R*V_R)$], and the digested mass going out of the aerobic digester (EAD). The aerobic digestion process is according to the illustration in Figure 3.7.

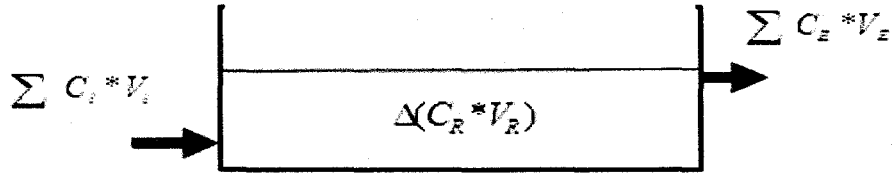


Figure 3.7: Solids mass balance [294]

In Figure 3.7, C_i is the concentration (mg/L) of MLSS/MLVSS IAD, V_i is the daily volume (L) of feed sludge, C_R is the concentration (mg/L) of MLSS/MLVSS in i-SGBR AD-C, V_R (L) is the AD-C volume (L), $\Delta C_R * V_R$ is the i-SGBR sludge solids mass (mg/d) over the sampling period, C_E is the EAD concentration (mg/L) MLSS/MLVSS, and V_E is the daily discharged volume from the EAD.

The solids destroyed (MLSS and MLVSS) and % solids destroyed for each digestion period were determined according to Equation 3.2 and Equation 3.3, respectively.

$$Solids\ destroyed = \sum(C_i V_i) - \sum(C_E V_E) - (\Delta C_R V_R) \tag{3.2}$$

$$\%\ Solids\ destroyed = \frac{Solids\ destroyed}{(C_i V_i)} \times 100\% \tag{3.3}$$

AD-C was operated and monitored based on 10 days SRT. The HRT during each period of monitoring of the AD-C was 24 hrs on the intermittent feed of only the RAS. Hence, with the AD-C volume of 75 L, 7.5 L of RAS concentrated sludge was fed daily into it and 7.5 L withdrawn. The AD-C valve was manually operated to be consistent with the required daily target volume, then valve closed as soon as pumping was completed. Since the AD-C volume and SRT were fixed, the daily sludge fed to the AD-C was obtained according to Equation 3.4

$$\theta_c = \frac{V}{Q}, (days) \tag{3.4}$$

Where *V (L)* is the aerobic digester volume, Q is the flow to the *AD-C*, and Θ_C is the solids retention time (days).

SRT was maintained through daily wasting of sludge from i-SGBR *WAS* point to get rid of excess growth. Wasting of sludge in i-SGBR was done according to Equation 3.5. The procedure to perform daily wasting in the ASP based on functional relationships have been detailed in previous researches [65, 84, 268]. The wasting was necessary to get rid of inert matter that might be non-biodegradable [291].

$$\theta_c = \frac{VX}{X_r Q_w} = \frac{Solids\ in\ the\ system,(kg)}{Wasting\ rate,(\frac{kg}{d})},(d) \tag{3.5}$$

In Equation 3.5, θ_C is the SRT, being the time biomass was kept in the system before being wasted [268], V is volume of *AER-C* (L), Q_w is waste sludge flow rate, *(L/d), X* and X_r are the *MLSS* concentrations (mg/L) in *AER-C* and *RAS*, respectively. The SRT was a function of solids in the system divided by the wasting rate [84, 268]. Equation 3.5 was used to determine various sludge wastage Q_w to maintain the SRT, with the known AER-C MLSS concentration, known volume of AER-C, and known RAS concentration (X_r). The mass of underflow was determined using Equation 3.6.

$$Under\ flow\ mass = X_r Q_w \left(\frac{mg}{d}\right) \tag{3.6}$$

In Equation 3.6, X_r and Q_w have been defined in Equation 3.5.

Operation of return activated sludge was performed with sludge returned to the AER-C through the ANX-C of the i-SGBR system, which is typical of anoxic/aerobic system (A/O). This was to maintain the biomass concentration for operation. This was achieved by determining the operational RAS ratio (R_i). Whereas consistency was maintained for return rate during each phase and stage of the operation. The RAS flow to maintain the biomass concentration in the AER-C depends on the RAS concentration. Therefore, the required biomass concentration in the AER-C was estimated based on Equation 3.7. The RAS pump was calibrated to achieve the needed concentration desired, considering factors such as the viscousity of the biomass. The sludge digested in the AD-C was intermittently emptied into the AER-C after its required HRT.

$$R_i = \frac{X}{(X_r - X)} \tag{3.7}$$

In Equation 3.7. X is the AER-C biomass concentration, mg/L, and X_r is the sludge concentration in RAS.

The RAS pump capacity was between 1-5 L/m, and the time to return the sludge to the AER-C was automatically phased into 8-time slots per day. Timer with idle and working modes was programmed to trigger and deactivate each action. Illustration of the procedure is according to Table 3.2. The flow was maintained by a control valve. The EAD was considered part of the biomass constituting recharge to AER-C. The RAS was operated between 50-100 % of the influent flow rate during the experiment.

Table 3.2: Time phase for the RAS recycle.

Description	Time slots															
RAS pumping	Slot 1		Slot 2		Slot 3		Slot 4		Slot 5		Slot 6		Slot 7		Slot 8	
	W	I	W	I	W	I	W	I	W	I	W	I	W	I	W	I

Note: W = working time mode, I = idle time mode

IR pump was used to transfer nitrate from AER-C to ANX-C. Nitrate produced in the AER-C as a result of oxidation of organic matter must be returned to the ANX-C to be reduced to nitrogen gas through the anoxic process. IR pump was used, which has the capacity to supply between 10-20 L/m. IR was operated for a number of 12 cycles per day during various operations of the OLR. The discretion of the interval for IRQ was to ensure regular and organized supply of nitrate to ANX-C.

3.4.2.2 Phase II performance monitoring of the system

Performance of i-SGBR system was monitored for removal of the organic matter (COD, sCOD, and BOD_5), nitrogen (ammonia-nitrogen, nitrate-nitrogen, TKN, and TN), total phosphorus, and TSS. Sludge degradation was evaluated based on MLSS and MLVSS reduction in the AD-C system. Experimental scheme for the phase II operation (II-A, II-B, II-C, and II-D) is presented in Table 3.3.

Table 3.3: Experimental scheme and i-SGBR operational conditions for phase II

Phase	II			
Experimental sub-phase	II-A	II-B	II-C	II-D
Experiment period (days)	1-50	52-87	89-131	134-162
Flow rate, Q_{INF}, (L/d)	45	45	70	100
Hydraulic retention time Θ_{Total}, HRT (days)	5.89		3.79	2.65
Organic loading rate, (OLR), AER-C	OLR_{AER}-1		OLR_{AER}-2	OLR_{AER}-3
Anoxic detention time, Θ_{ANX-C}, (days)	0.888		0.571	0.40
Aeration detention time, Θ_{EA-C}, (days)	2.78		1.786	1.25
Aerobic digester detention time, Θ_{AD-C}	-	24 hrs		
Clarifier detention time, Θ_{CLAR}	2.22		1.43	
Solids retention time Θ_c, SRT (days)	>40	40	40	40
Solids retention time Θ_c, SRT_{AD-C} (days)		10		
Internal recycle ratio, IR	3	6	6 & 3 (Monitoring effects of IR on denitrification)	IR_3
Internal recycle flow, Q_{INF} *IR, (L/d)	135	270	420 & 210	600
Vol. Pumped in each cycle (12 cycles), (L)	11	23	35 / 18	Optimum ratio from II-C
Pumping duration, (minutes)	1.0	2.0	2.5 / 1.5	
MLSS in AER-C, mg/L	Build up to 3000	3000	3000	3000 - 5000
RAS ratio, Ri = [X/(Xr-X)]	R_1		R_2	R_3
Total RAS flow (R_{Total}) = (R_i) * Q_{INF}, (L/d)	R_1 * 45		R_2 * 70	R_3 * 100

Phase II-A: At the stage of the start-up, leakages and air tightness were checked in i-SGBR system, then volume of 130 L activated sludge from beverage WWTP.

Figure 3.8 a. and b. show an extended aeration tank and RAS storage pit. Sludge for seeding comprise 150 L from plant's extended aeration tank 1 (EA-T) (Figure 3.8 a), and 50 L from the plant's RAS storage pit was allowed to settle and used for inoculation of i-SGBR (Figure 3.8 b).

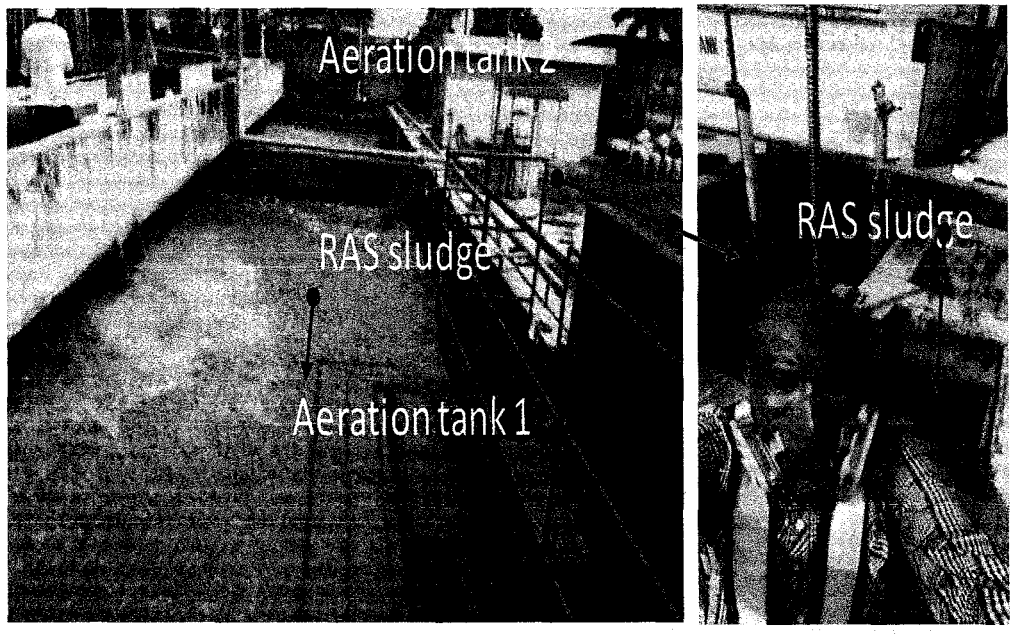


Figure 3.8: (a). Extended aeration tank and (b). RAS storage pit

The sludge samples were left to settle down during initial 30 minutes, before charging settled sludge volume of 130 L inside i-SGBR bioreactor. Common procedure was described in previous research [296]. The aeration DO was set between 3-5 mg/L and 6 – 10 mg/L in AER-C and AD-C, respectively. This was to ensure provision of sufficient mixing and suspension of biomass. The continuous feed was maintained at 45 L/d and closely monitored for effluent and biomass quality.

Sludge sample from the EA-T and RAS prior to seeding were measured to determine the quality of its properties. Parameters such as SSV, SVI, pH and RAS were measured. The RAS (Xr) concentration was evaluated from both MLSS and SVI parameters [85, 91, 95]. The MLSS and SSV in EA-T and RAS, were 3,188 mg/L and 250 mL/L, and 15,800 mg/L and 850 mL/L, respectively. The SVI for the EA-T (tank 1) was 78.4 mL/g. The pH of sludge samples was in the range of 6.2-6.8.

In phase II-A, the i-SGBR system was operated between day 1-50 with Q of 45 L/d. There was no wasting of sludge in the i-SGBR system prior to reaching MLSS concentration of 3,000 mg/L, however, wasting began when desired concentration was reached. MLSS for the operation was 3,000 mg/L. The IRQ was set for 135 L/d corresponding to IR ratio of 3, and RAS ratio of 0.8 corresponding to 36 L/d were regulated during the period of the start-up. Sludge was wasted between day 32-50 after the biomass build-up to maintain SRT of 40 days, and IR ratio set to 6 corresponding to IRQ of 270 L/d. ANX-C and AER-C HRT were 0.88 day and 2.78 days, respectively.

Phase II-B: This phase was operated between day 52-87 with Q of 45 L/d, and monitoring of sludge digestion started in phase II-B. AD-C was seeded with 75 L and 25 L sludge from the EA-T and RAS pit on day 49. The aeration was set between 6-10 mg/L. The AD-C operation started on day 50. The AD-C influent was supplied from i-SGBR RAS. SRT operated for the i-SGBR system and AD-C were 40 days and 10 days, respectively. The RAS ratio to be operated will be adjusted according to the sludge concentration in the CLR and the target concentration in the AER-C. Although, RAS flow was set as the product of Q (L/d) and the RAS ratio. HRT for phase II-B for ANX-C and AER-C were 0.88 day and 2.78 days, respectively. The operational MLSS was 3,000 mg/L. IRQ was set for 270 L/d corresponding IR ratio of 6.

Phase II-C: This phase was operated between day 89-131 with Q of 70 L/d and simultaneous operation of aerobic digestion. In this phase, IR ratio of 6 and 3 was varied to monitor denitrification performance. IR ratio of 6 and 3 correspond to IRQ of 420 L/d and 210 L/d, maintained between day 89-110, and between day 113-131, respectively. The shift in IR of 6 to 3 was done after each preceding steady state of 6 was attained. RAS ratio operated was according to the sludge concentration in the CLR, and the target concentration in AER-C, where the RAS flow was set as a product of Q (L/d) and the RAS ratio. The ANX-C and AER-C HRT were 0.57 day and 1.79 days, respectively. The operational MLSS was 3,000 mg/L. i-SGBR and AD-C SRT operated were 40 days and 10 days, respectively. The IRQ was set for 420 L/d corresponding IR ratio of 6.

Phase II-D: Operation in this phase was carried out between day 134-162 with Q of 100 L/d and operation of simultaneous aerobic sludge digestion. SRT operated for the i-SGBR system and AD-C were 40 days and 10 days, respectively. Conversely, the actual daily wasting of sludge was performed include the efficiency derived from the aerobic digester MLSS degradation efficiency. The ANX-C and AER-C HRT were 0.40 and 1.25 days, respectively. The operational MLSS for this phase was 3,000 mg/L. Optimum IR ratio from phase II-D will be chosen. The RAS flow will be determined according to the MLSS concentration in the AER-C and RAS concentration. The AER-C MLSS was gradually build-up from 3,000-5,000 mg/L between days 155 to 162 without sludge wasting and gradual addition of sludge.

3.4.3 Phase III Experimental scheme for operation

This phase III was operated between day 164-278. The purpose was to determine the bio-kinetics parameters for the carbon oxidation, which was operated with five flow rates; 100, 110, 120, 135 and 150 L/d. Simultaneous operation of sludge digestion was monitored with SRT of 10 days. Sludge degradation was evaluated based solids reduction. Parameters such as COD, sCOD, and BOD_5, ammonia-nitrogen, nitrate-nitrogen, TKN and TN, total phosphorus, TSS, MLSS, MLVSS, SVI, pH, temperature, DO and food to micro-organisms (F/M) ratio were measured. The volumes of ANX-C, AER-C, AD-C and CLR were fixed throughout the duration of the experiment.

3.4.3.1 Phase III operation and control parameters

System operation and control parameter such as MLSS, MLVSS, SVI, pH, temperature, DO and food to micro-organisms (F/M) ratio were monitored. The i-SGBR system was operated for 40 days SRT and AD-C for 10 days SRT. Consequently, other control parameters such as MLSS, MLVSS, SVI, pH, temperature, DO and food to micro-organisms (F/M) ratio were monitored. Microbial parameters such as heterotrophic plate count (HPC) and determination of microfauna such as protozoa and metazoa was

carried out from the sludge samples in the aeration chamber. i-SGBR system was operated for 40 days SRT and AD-C for 10 days SRT as discussed in Section 3.4.2.1.

3.4.3.1 Phase III performance monitoring of the system

Monod's model was adopted to determine the biokinetic coefficients. The process to determine the bio-kinetic parameters according to Monod's model requires operating five different OLR of extended aeration detention times; SRT (20-40 days) and HRT (20-30 hours), and operational AER-C MLSS (5,000 mg/L). In this phase, MLSS concentration of 5,000 mg/L was chosen and fixed for the operation. IRQ to be operated in this phase was based on optimum IR ratio obtained from phase II-D.

In phase III, details of five consecutive activities operated include the following;

i. Phase III-A was operated between day 164-185 with Q of 100 L/d, ANX-C and AER-C HRT of 0.4 days and 1.25 days, respectively, i-SGBR an AD-C SRT were 40 days and 10 days, respectively.

ii. Phase III-B was operated between day 187-208, with Q of 110 L/d, ANX-C and AER-C HRT of 0.36 day and 1.14 days, respectively, i-SGBR an AD-C SRT were 35 days and 10 days, respectively.

iii. Phase III-C was operated between day 211-232, with Q of 120 L/d, ANX-C and AER-C HRT of 0.33 day and 1.04 days, respectively, i-SGBR an AD-C SRT were 30 days and 10 days, respectively.

iv. Phase III-D was operated between day 234-255 with Q of 135 L/d, ANX-C and AER-C HRT of 0.30 day and 0.96 days, respectively. i-SGBR and AD-C SRT were 25 days and 10 days, respectively, and

v. Phase III-E was operated between day 257-278 with Q of 150 L/d, ANX-C and AER-C HRT of 0.27 day and 0.83 days, respectively. i-SGBR and AD-C SRT were 20 days and 10 days, respectively.

Parameters and conditions for the experimental scheme in the phase III operation drawn for this Section is as provided according to items in Table 3.4.

Table 3.4: Experimental scheme and i-SGBR operational conditions in phase III

Phase	III				
Experimental stage	III-A	III-B	III-C	III-D	III-E
Experimental period (days)	164-185	187-208	211-232	234-255	257-278
Flow rate, Q_{INF}, (L/d)	100	110	120	135	150
Hydraulic retention time Θ_{Total}, HRT (days)	2.65	2.41	2.20	2.00	1.77
Organic loading rate, (OLR), AER-C, (kg COD/m^3. d)	OLR_{AER-3}	OLR_{AER-4}	OLR_{AER-5}	OLR_{AER-6}	OLR_{AER-7}
Anoxic detention time, Θ_{ANX-C}, (days)	0.40	0.36	0.33	0.30	0.27
Aeration detention time, Θ_{AER-C}, (days)	1.25	1.14	1.04	0.96	0.83
Aerobic digester detention time, Θ_{AD-C}, (days)	24 hrs				
Clarifier detention time, Θ_{CLAR}, (days)	1.00	0.91	0.83	0.74	0.67
Solids retention time Θ_c, SRT_{i-SGBR} (days)	40	35	30	25	20
Solids retention time Θ_c, SRT_{AD-C} (days)	10				
Internal recycle ratio, IR	6				
Internal recycle flow, Q_{INF} *IR, (L/d)	600	660	720	810	900
MLSS in AER-C, mg/L	5000				
RAS ratio, Ri = [X/(Xr-X)]	R_3	R_4	R_5	R_6	R_7
Total RAS flow (R_{Total}) = (R_i) * Q_{INF}, (L/d)	R_4 *100	R_5*110	R_6*120	R_7*135	R_8*150

Steady state data during each loading was generated to determine the biokinetics coefficients. In this phase, removal performance based on HRT was also evaluated for COD, BOD_5, TSS, ammonia-nitrogen, nitrate-nitrogen, TKN, and TN.

Wastewater used in phase III operation was diluted, where average value and standard deviation of 1,019±8.8 mg/ was obtained as the influent COD of the wastewater. This was to ensure regular organic loading into i-SGBR system during biokinetic study. Initial COD of sample was always determined and dilution was done according to Equation 3.8 as follows:

$$C_1V_1 = C_2V_2 \quad (3.8)$$

Where, C_1 (mg/L) is concentration of the raw wastewater prior to dilution, V_1 (L) is volume for dilution (unknown), C_2 is the target concentration of 1,000 mg/L, and V_2 is the working volume, which ranges between 100–150 L. Wastewater reserve above 100 L was constantly kept as back up in cold room, should subsequent influent concentration were to be below-required concentration. Reserves are stored for 2 days at 4 °C before being disposed and replaced.

Various suspended growth process models have appeared in the wastewater treatment literature [297-299]. Common parameters such as BOD_5, COD, and NH_4^+-N were used as a substrate for the determination of the biokinetic coefficients, on the assumption that the removal was exclusively due to aerobic biodegradation [300].

In a continuous culture systems, the growth of bacteria cells and effect of limiting substrate or nutrient normally can be expressed according to Equation 3.9 and Equation 3.10, respectively [301] as follows:

$$r_s = \mu X \quad (3.9)$$

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad (3.10)$$

Where in Equation 3.8, r_g is the bacterial growth (mg/L. d), μ is the specific growth rate (d^{-1}), and X is the biomass concentration (mg/L). In Equation 3.9 μ_{max} is the maximum specific growth rate (d^{-1}), S is the concentration of growth limiting substrate (mg/L), K_s is the saturation constant which is equivalent to 0.5 μ_{max} (mg/L), and μ is the specific growth rate, (d^{-1}).

The model was developed based on the following assumptions;

i. bioreactor is completely mixed with aerators installed at the bottom of its tank.

ii. influent substrate concentration remains constant.

iii. no microbial solids are contained in the influent substrate.

iv. the volume of the bioreactor is constant.

v. complete rejection of MLSS (no biomass allowed in the effluent).

vi. the substrate is not rejected, and a steady state exists throughout the system.

Equation 3.11 and Equation 3.12 are the linear relationships between biomass and substrate, respectively. The data during each steady state phase was reported as average and standard deviation to evaluate bio-kinetic co-efficients [84, 268, 280, 302].

$$\frac{1}{\theta c} = YU - K_d = Y\left(\frac{So-S}{\theta X}\right) - K_d \quad (3.11)$$

$$\frac{1}{U} = \frac{X\theta}{So-S} = \left(\frac{Ks}{k}\right)\left(\frac{1}{S}\right) + \frac{1}{K} \quad (3.12)$$

The linear regression plots were used to determine bio kinetic coefficients using Microsoft excel 2013. In Equation 3.11 and Equation 3.12, θ_C is the solids retention time (d), Y is biomass yield coefficient (mg VSS/mg sCOD), U is substrate utilization rate, (mg sCOD/mg VSS. d), K_d is endogenous decay coefficient (d^{-1}), S_O is the influent substrate concentration (mg sCOD/L), S is effluent substrate concentration (mg sCOD/L), X is biomass concentration (mg VSS/L), Θ is hydraulic retention time (hours), Ks is substrate concentration at half the maximum growth rate (mg sCOD/L), and k is maximum rate of substrate utilization (mg sCOD/mg VSS d).

In Equation 3.11, plot of $1/\theta_C$ vs. U will yield Y and K_d as slope and intercept, respectively. Whereas, plot of $1/U$ vs. $1/S$ in Equation 3.12, yields Ks/k and k as slope and intercept, respectively. k_s is estimated with k as a known value. This equation can be applied in case of system with recycle or non-recycle of biomass [303, 304].

The maximum specific growth rate, μ_{max} (d^{-1}), was also determined by multiplying coefficients k and Y according to Equation 3.13.

$$\mu_{max} = kY \tag{3.13}$$

3.5 Analytical procedure and sampling

The analytical methods, procedures and laboratory equipment used in this research are detailed here. The analysis was done in accordance with the procedure in the standard methods for the examination of water and wastewater, 21st edition [305]. The in situ analysis were carried at pilot study site, while major analysis at environmental laboratory of the Universiti Teknologi PETRONAS. Once the samples were obtained from the bioreactor, they were preserved and analysed in the laboratory. Samples were centrifuged immediately at 1,000 rpm for 10 minutes to halt biological activity. Nonetheless, all samples collected were analyzed the same day.

The samples analyzed include physio-chemical parameters such as the COD, BOD_5, ammonia-nitrogen, nitrate-nitrogen, total phosphorus, color, TSS, TKN, MLSS and MLVSS were carried at the laboratory of the University Teknologi PETRONAS, while parameters like DO, temperature and pH were measured in situ at the pilot study site. However, necessary precautions of preserving the sample prior to analysis were always observed. This includes sealing the cap bottled samples in a polyethene bag and storing in a cooler. The pilot study site to Univeristi Teknologi PETRONAS laboratory is 15 km. The microbiological parameters practiced for the activated sludge include heterotrophic plate count (HPC) as a measure for testing of bacterial population, and detection of protozoa and metazoa as microfauna were carried out based on random testing from grab samples in the aeration chamber.

The summary of the specific tests and sampling points is as demonstrated in Table 3.5. Total COD was measured at five sampling points; influent, E-ANX-C (corresponding to influent AER-C), E-AER-C (corresponding to IR), EAD, IAD (influent aerobic digester chamber) (corresponding to RAS), and E-CLR. The BOD_5, total phosphorus, ammonia-nitrogen, total nitrogen, TSS, and TKN were measured from the influent and effluent samples only. The samples for HPC and microfauna were done for samples in the aeration chamber. The nitrate was measured from five sampling

points, which include; influent, E-ANX-C, E-AER-C, EAD, and E-CLR. Samples for MLSS and MLVSS were obtained from the bio-reaction chambers in ANX-C, AER-C, AD-C, and CLR.

Table 3.5: Specific tests for each sampling point

Parameter	Method	Testing Location	I	ANX-C	AER-C	IAD	EAD	E
			Bio-reactor Sampling locations points					
1). COD	Colorimetric	UTP	X	X	X	X		X
2). sCOD	Colorimetric	UTP	X	X	X			X
3). BOD_5	Membrane electrode, 5 days incubation at 20 °C	UTP	X					X
4). Total Phosphorus	Colorimetric	UTP	X					X
5). Total Nitrogen	Persulphate digestion	UTP	X					X
6). Ammonia-N	Nessler method	UTP	X					X
7). Nitrate	Cadmium reduction	UTP	X	X	X			X
8). Nitrite	Diazotization LR	UTP						X
9). TKN	Simplified TKN	UTP	X					X
10). MLSS/ MLVSS	Gravimetric	UTP		X	X	X		
11). TSS	Gravimetric	UTP	X					X
12). pH & Temperature	Electrode	Site	X		X	X		X
13). DO				X	X	X		
14). HPC	3M Petrifilm Aqua Plate Testing Process	UTP			X			
15). Microbial observations	Counting under microscope	UTP			X			

Notes: I = Influent, ANX = Pre-anoxic, AER = Aeration, IAD = Influent Aerobic digester, EAD = Effluent Aerobic digester, E = Effluent Clarifier, BOD_5 = Five days biochemical oxygen demand, COD = Chemical oxygen demand, pH = Potential of hydrogen, MLVSS = Mixed liquor volatile suspended solids, MLSS = Mixed liquor suspended solids, DO = Dissolved oxygen. Units in mg/L, except pH, and temperature in °C, HPC = Heterotrophic plate count

Relevant reagents, consumables and some equipments including spectrophotometer DR 3900 series (HACH brand) were purchased from ARACHEM (M) Sdn. Bhd. (127181-D) Kuala Lumpur, Avantis laboratories Sdn. Bhd, Ipoh, and Era Bumi Sains Sdn. Bhd. (1054116-P) Selengor, Malaysia.

These samples were collected to evaluate removal efficiencies and to determine performance of nitrification, denitrification, specific nitrification and denitrification rates, and determination of the bio-kinetic co-efficient, which was carried out from soluble COD and MLVSS samples. Sampling was performed three times per week, where each sample was collected and analyzed in triplicates. The operation and control parameters such as DO, temperature and pH were checked daily. Illustration of the site sampling from bioreactor is as shown in Figure 3.9a. During this study, batch samples for the continuous flow i-SGBR system were obtained from the designated ports, according to the illustration provided for the processes in Figure 3.9b.

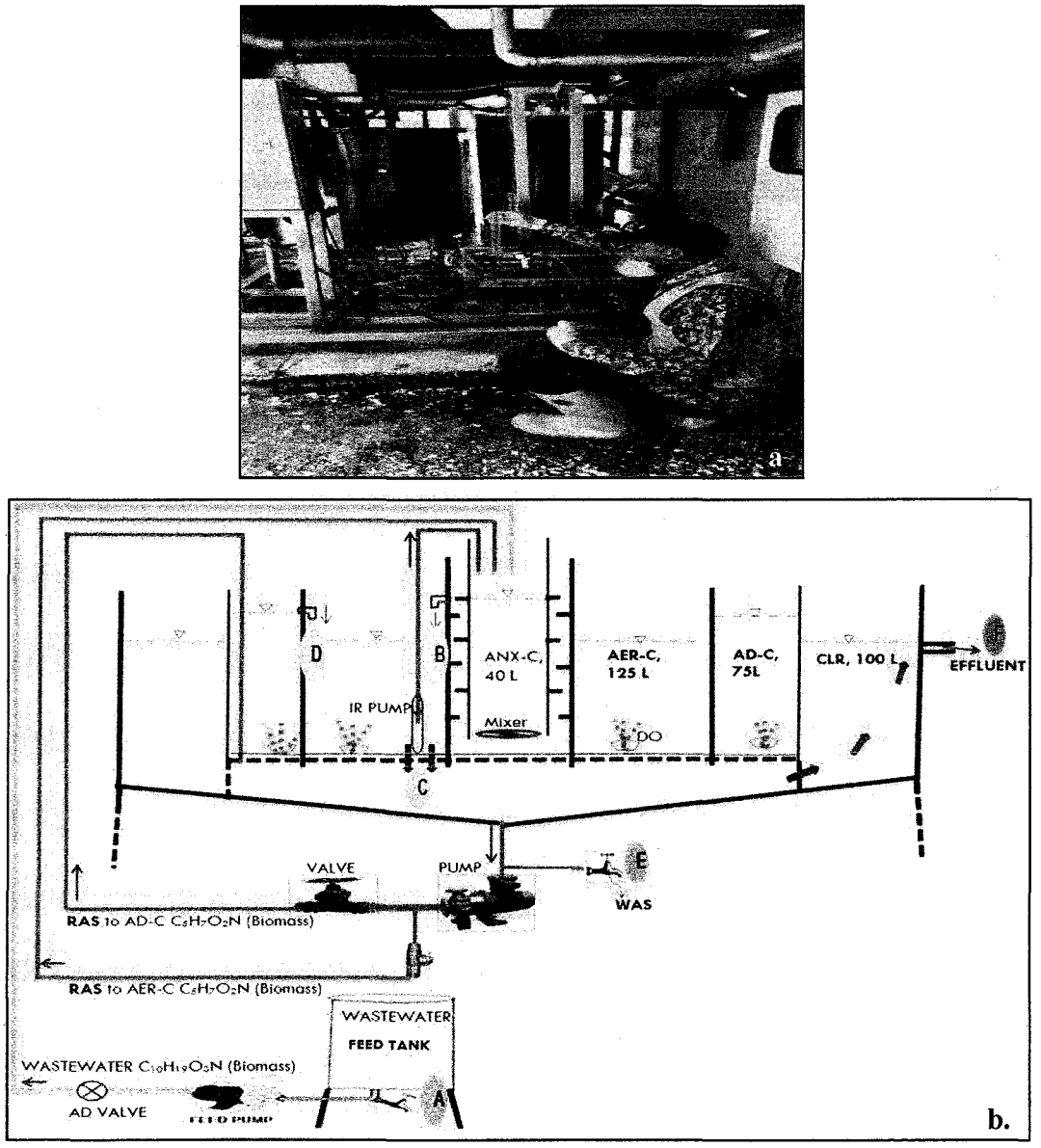


Figure 3.9: i-SGBR sampling points A, B, C, D, E, and Site sampling

These points consist of influent (A), E-ANX-C (B), E-AER-C (C) (corresponding to IR), EAD (D), IAD (E) (corresponding to RAS), and E-CLR (F), respectively. Colorimetric tests were used to determine COD, Total nitrogen, Ammonia-nitrogen, Total Kjeldahl nitrogen (TKN), Nitrate-nitrogen, and Total phosphorus. These tests were performed using spectrophotometer HACH DR 3900 and digestion vessel DRB200 (Figure 3.10), vial racks, 10/20 mL cuvettes, micro fiber glass filter paper to determine the soluble samples, and specific reagents for individual tests explained in the analytical procedure.

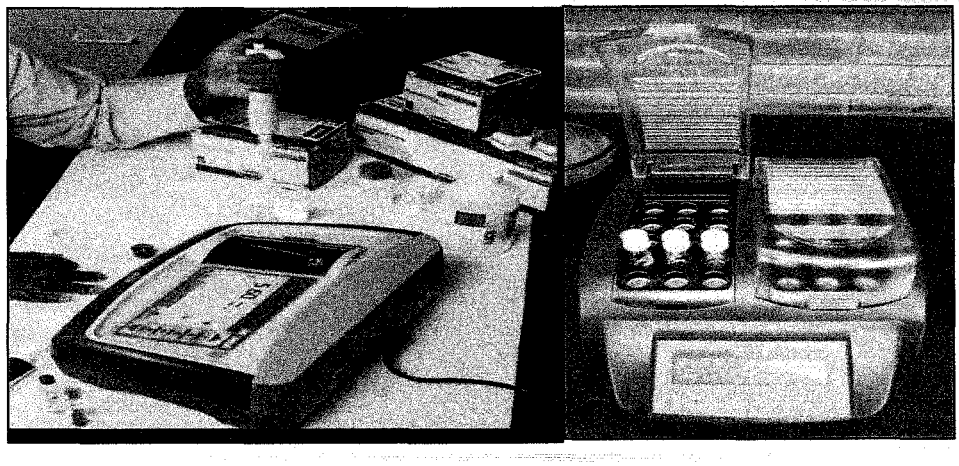


Figure 3.10: DR 3900 spectrophotometer and DRB 200 digestion block

3.5.1 Measurement of Chemical oxygen demand (COD)

The Chemical Oxygen Demand (COD) (mg/L) is defined as the amount of a quantified oxidant that reacts with the sample in a regulated condition. The quantity of oxidant consumed is expressed in terms of its oxygen equivalent. Because of its unique chemical properties, dichromate ion ($Cr_2O_7^{2-}$) is normally the particular oxidant used.

The procedure starts with a 100 mL of sample was homogenized for 30 seconds in a blender. The DRB200 Reactor need to be turned on and preheat was set to 150 °C. The caps were removed from two COD Digestion Reagent Vials. A clean volumetric pipet was used to add 2 mL of sample to the vial. Another clean volumetric pipet was used to add 2 mL of distilled water to the vial for the blank sample. Cap of the vials were closed tightly and the vials were shaken vigorously. The sample vials become very

hot during mixing. The vials were heated for two hours using the DRB200 reactor. The vials were placed into a rack and cool to room temperature. The vials were wiped with a damp towel followed by a dry one. The blank vial sample was put into the spectrophotometer in order to set it to zero. Then the sample vial was put into spectrophotometer to record the COD reading in mg/L.

To obtain the COD for the soluble COD (sCOD), the samples were first filtered with Whatman cellulose nitrate membrane filters (0.45 μm, Ø 47 mm), and above steps repeated. Finally, all COD readings were recorded.

3.5.2 Measurement of Biochemical oxygen demand (BOD_5), pH, Temperature and Dissolved oxygen

The Biochemical Oxygen Demand (BOD) (mg/L) is a measure of the quantity of the oxygen consumed by bacteria when stabilizing degradable organic matter under aerobic conditions according to Equation 3.14:

$$Organic\ matter + DO \xrightarrow{Bacteria} CO_2 + Biological\ growth \qquad (3.14)$$

The BOD samples were analyzed at a temperature of 20 °C and 5 days of incubation stated in method 5210-B, Standard Methods for the Examination of Water and Wastewater [305]. 19 L of aerated water was prepared one day before the experiment was conducted, by using a diffuser that was placed inside the water container to supply oxygen at 2.0 mg/L. After the aerated water was prepared, BOD buffer nutrient was poured into the 19 L of aerated water and waited for 30 minutes reaction time. The blank sample was prepared by pouring aerated water into a 300 mL BOD bottle until it reaches the neck. The dilution made follows Table 3.6, conversely, specific amount of wastewater sample was taken and poured inside the BOD bottle and filled up with aerated water until it reaches its neck. Blank sample was measured with DO meter and the reading was recorded. The BOD bottle was closed with cap and aluminum foil in an air tight manner. The BOD bottles were kept inside the BOD incubator, where the temperature was set at 20 °C, and stored for 5 days.

Table 3.6: Dilution range by direct pipetting into 300-mL bottles [84]

Volume of sample (mL)	Range of BOD, mg/L	
	Maximum	Minimum
1.0	600	2,100
2.0	300	1,050
5.0	120	420
10	60	210
20	30	105
50	12	42
100	6	21
300	0	7

After 5 days, the BOD bottles were measured by using DO meter and readings recorded. The initial and final DO of the diluted sample should not be less than 2.0 mg/L, final DO should not be less than 1 mg/L, the blank was only distilled water with no microorganisms, the difference in initial and final DO for blank sample should not exceed 0.2 mg/L, the blank correction must be applied for each prepared sample, and finally the final DO reading must be recorded at room temperature. The BOD_5 was evaluated based on Equation 3.15.

$$BOD_5 = \frac{(DO_i - DO_f)_{sample} - (DO_i - DO_f)_{Blank} \times F}{P} \quad (3.15)$$

Where, DO_i and DO_f are the initial and final dissolved oxygen concerntrations of the diluted and blank samples, respectively. P = decimal volumetric fraction of sample used (volume of sample used in 300mL / 300 mL), and F is the BOD factor (300 mL – dilution volume)/300 mL.

The temperature was not controlled, however, the temperature in the AER-C was monitored daily through out the duration of the experiments. Values of pH were measured using a pH meter. Addition of sodium bicarbonate was ensured to keep buffer for maintaining pH close to neutral. pH and temperature of the samples were measured immediately using potable meters, HACH pH Sensor +, PH3 and probe.

Dissolved oxygen is required for the respiration of aerobic microorganisms. The minimum DO concentration of 1 to 2 mg/L in the aerobic systems was maintained to prevent anaerobic/anoxic conditions to exist in the i-SGBR system. Portable DO probe, model YSI 550 A was used for measurement of DO in both AER-C and AD-C.

The BOD_5 was determined by membrane electrode method, using Yellow Spring Instrument (YSI) DO meter YSI 5100. These equipment are labeled in Figure 3.11 as pH and BOD meters, BOD incubator, and portable DO meter.

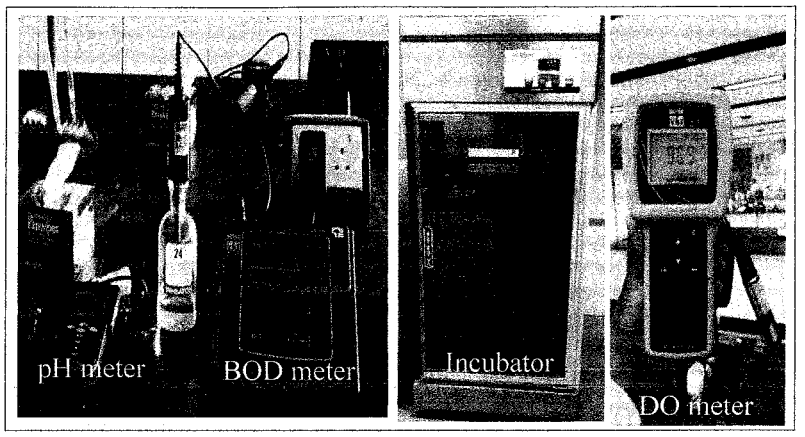


Figure 3.11: pH & BOD meter, incubator, and DO meter

3.5.3 Measurement of Total nitrogen

The Total nitrogen (TN) (mg/L) was analyzed by Persulfate Digestion Method 10072. The measurement range was 2 to 150 mg/L N (HR). The process commenced with starting the DRB200 Reactor and setting the temperature to 105 °C. A funnel was used to add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two HR Total Nitrogen Hydroxide Digestion Reagent vials and cleaned. Then 0.5 mL of sample was added to one of the vials . Another 0.5 mL of deionized water was added to the second vial as blank. The caps on both vials were closed and shook vigorously for 30 seconds to mix. The undissolved powder will not affect the accuracy of the test.

The vials were placed in a reactor to digest for exactly 30 minutes. The vials were removed and allowed to cool at room temperature for 30 minutes. The contents of one Total Nitrogen (TN) Reagent A Powder Pillow was added to each vial. The caps on both vials were placed tight and shook for 30 seconds. The instrument timer was started, and 3-minutes reaction time started. After the timer expires, the caps from the vials were removed. Then one TN Reagent B Powder Pillow to each vial was added, and the caps on both vials were put and shook vigorously for 15 seconds to mix. The reagent will not dissolve completely. Undissolved powder will not affect accuracy of the test.

The solution will start transforming to yellow. The instrument timer was started, and 2 minutes reaction time starts. When the timer expires, a pipet was used to put 2 mL of the digested, treated prepared sample into one TN Reagent C vial, followed by 2 mL of the digested, treated blank into the second TN Reagent C vial. The caps on both vials were put and inverted 10 times to mix. Slow, deliberation inversions for complete recovery were ensured. The vials will be warm to the touch. The instrument timer was started and 5-minutes reaction time starts. The yellow color will intensify. When the timer expires, the blank vial was cleaned. Then blank vial was inserted into the 16-mm cell holder and zero was pressed (display shows 0 mg/L N). Sample vial was cleaned and placed inside the 16-mm cell holder and the final reading obtained in mg/L N.

3.5.4 Measurement of Ammonia - nitrogen

The USEPA Nessler Method (Method 8038) was used to determine the ammonia nitrogen (mg/L). The spectrophotometer has detection range between 0.02 to 2.50 mg/L NH_3^+–N. Sample and blank were prepared by filling 25 mL of sample and deionized water into separate mixing cylinder.

Three drops of Mineral Stabilizer was then added to both mixing cylinders and mixed thoroughly. The Mineral Stabilizer serves to break the complex hardness in the sample. Then, three drops of Polyvinyl Alcohol Dispersing Agent was added to each cylinder (to aid in the color formation for the reaction). This is followed by dropping 1.0 mL of Nessler Reagent to both sample and blank. The cylinders were inverted several times to ensure sufficient mixing. The mixture was allowed to react for one-minute. 10 mL of the mixture of each solution were poured into sample cells. The blank sample was used to zero the spectrophotometer before the sample to determine the ammonia- nitrogen was measured. The readings were recorded.

3.5.5 Measurement of Nitrate – nitrogen

The nitrate nitrogen (mg/L) was determined using Cadmium reduction method (Method 8039). The spectrophotometer has detection range between 0.3 to 30.0 mg/L NO_3–N

(HR). 10 mL of the sample was poured inside a sample. Then, the content of NitraVer 5 reagent was added using a mini funnel, shaken for one-minute, and allowed to react for five-minutes. An amber color developed if nitrate was present. The contents of nitrate can then be measured after the instrument was zero using the blank. The blank was prepared by filling the sample cell with 10mL of the sample.

3.5.6 Measurement of Total kjeldahl nitrogen (TKN)

The Total Kjeldahl Nitrogen (TKN) (mg/L) was analyzed using Simplified TKN (s-TKN) method 10242, TNTplus™ 880. The spectrophotometer measuring range was between (0 to 16 mg/L TKN. The procedure begins with powering on the DRB 200 reactor, and setting it to the temperature of 100 °C. Then, add 1.3 mL of sample, 1.3 mL of Solution A and 1 Reagent B tablet in quick succession to a dry 20-mm reaction tube. The reaction tube was closed immediately. The sample must not be inferred. The reaction tube was inserted in the preheated DRB200 reactor. The lid was closed and digested for 1 hour. The reaction tube was removed and allowed to cool at room temperature. 1 Micro Cap C is added to the reaction tube. The tube was well tightened, inverted to mix thoroughly. A pipette was used to add 0.5 mL of the digested sample from the 20-mm reaction tube into a test vial 1 (red label). Again, the pipette was used to add another 0.2 mL of Solution D to the test vial. The cap was quickly tightened on the vial and inverted until completely mixed. The next step was immediately continued, which was pipetting 1.0 mL of undigested sample to a test vial 2 (green label). This was followed by pipetting and the addition of 0.2 mL of Solution D to the test vial. The cap was quickly tightened on the vial and inverted until completely mixed. The reaction time of 15 minutes was started. The vials were wiped when the timer expired. The test vial 1 (red color) was inserted in the spectrophotometer 3900 into the cell holder to zero, with a display showing E1. The next step was to immediately insert test vial 2 (green label) into the cell holder and read. The results obtained will show in mg/L Total N, mg/L NO_3–N + NO_2–N and mg/L TKN.

3.5.7 Measurement of Total phosphorus

Total Phosphorus (TP) (mg/L) was measured by PhosVer® 3 Acid Persulphate Digestion Method (Method 8190) by USEPA. The procedure was started by preheating DRB200 reactor to 150 °C. Then, 5 mL of sample is poured into TP Vial using micro pipette after filtration. The contents of one potassium persulfate powder pillow are emptied inside the vial, using a mini funnel. The vial was capped tightly and shaken to dissolve the reagent. The vial is inserted inside the DRB200 to digest for 30 minutes. The vial was removed from the reactor and cooled to room temperature in the test tube rack, where 2 mL of 1.54 N sodium hydroxide standard solution is added inside the vial by using micro pipette. Through mixing was done for the solution by shaking, the vial is wiped and inserted in the Spectrophotometer to first zero the instrument. A mini funnel was used to add contents of PhosVer 3 powder pillow to the vial. The vial was tightly capped immediately and shaken to mix for 20-30 seconds. The powder will not dissolve entirely. Consequently, a timer was set for 2 minutes to allow for the reaction in the vial. Finally, after the timer expires, then the vial was inserted and reading recorded from the Spectrophotometer.

3.5.8 Measurement of Total suspended solids, mixed liquor suspended solids and mixed liquor volatile suspended solids

The TSS, MLSS, and MLVSS were determined by the gravimetric method. The suspended solids removal is considered to be essential when assessing the operational performance of wastewater treatment systems. Also, the concentration of biomass in the ANX-C, AER-C and CLR are represented by mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS).

Total and volatile suspended solids (mg/L) were determined following the reference method 2540D and 2540E, respectively [305]. The filter papers were prepared by letting a distilled water pass through to open the pores at least 24 hours prior to the test.

To start the procedure, a known volume of wastewater (V) was filtered through a Whatman 934-AH 47 mm glass fiber filter , which is 0.45 μm pore size, and weighed as *(W_1)*. The filter paper with the MLSS was maintained at 105 °C for 1 hour in an oven,

and placed in a desiccator to cool down for 10 minutes, and weighed *(W_2)*. The MLSS concentration was calculated according to Equation 3.16. To determine the MLVSS, the MLSS was maintained at 550 °C for 15 minutes in an electric muffle furnace, and introduced to a desiccator to cool for 10 minutes, and weighed *(W_3)*. MLVSS was calculated according to Equation 3.17.

$$TSS\ \left(\frac{mg}{L}\right) = \frac{W_2\ (g) - W_1\ (g)}{V\ (L)} \tag{3.16}$$

$$VSS\ \left(\frac{mg}{L}\right) = \frac{W_2\ (g) - W_3\ (g)}{V\ (L)} \tag{3.17}$$

The procedure to determine total suspended solids (TSS) (mg/L) in the influent and effluent is repeated same as the MLVSS process, except the paper to be used here was Whatman filter paper no. 1, 47 mm.

Equipments in Figure 3.12 were used in the determination of the TSS, MLSS and MLVSS. These equipments in Figure 3.12a are; vacuum suction pump, desiccator, digital analytical balance (MAX 200G) and oven (103–105 °C), while in Figure 3.21b is the electric muffle furnace (ashing at 550 °C). The filter papers used comprise of Whatman filter paper 934 AH, 47 mm, cat 1827-047 for MLSS and MLVSS, and Whatman filter paper no. 1, 47mm, cat 1001047 for TSS.

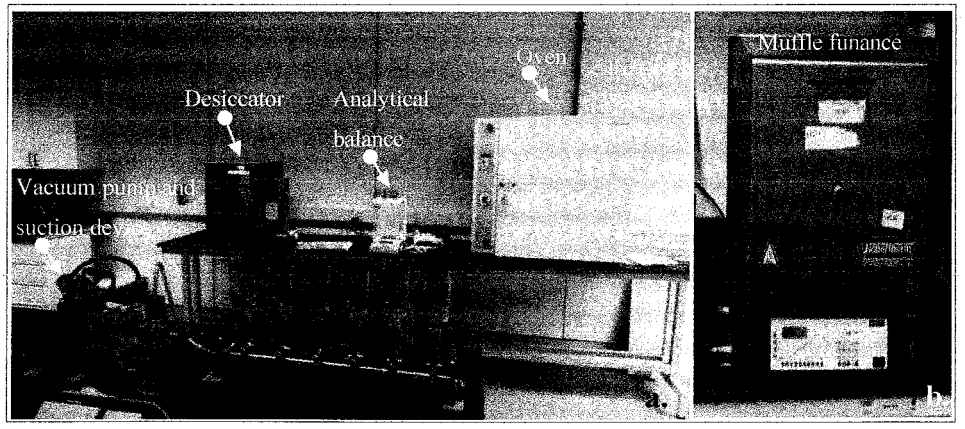


Figure 3.12: Desiccator, balance, vacuum pump, oven & muffle furnace

3.5.9 Measurement of Sludge volume index

The SVI was measured in the AER-C. The RAS (X_r) concentration was determined and evaluated from both MLSS and SVI parameters [85, 91, 95], according to Equation 3.18 and Equation 3.19, respectively. The calculated X_r was validated with the laboratory gravimetric measurements for MLSS.

$$SVI = \frac{SSV\left(\frac{mg}{L}\right) \times 1000}{MLSS\left(\frac{mg}{L}\right)}, \left(\frac{mL}{g}\right) \tag{3.18}$$

$$X_r = \frac{10^6}{SVI}, \left(\frac{mg}{L}\right) \tag{3.19}$$

Where, parameters in Equation 3.18, SSV is the settled sludge volume, while other parameters have already been defined earlier in Section 2.6.

3.5.10 Measurement of microbial heterotrophic plate count and determination of microfauna

The measurement to ascertain the bacterial population were enumerated using heterotrophic plate count (HPC), and the population of the microfauna from the sludge sample was identified using an optical microscope equipped with computer monitor (Leica DM LB2, Japan). The samples were obtained from the aeration chamber.

3.5.10.1 Measurement of heterotrophic plate count

The Heterotrophic Plate Count (HPC), otherwise known as the aerobic plate count or standard plate count, is a procedure for estimating the number of live heterotrophic bacteria in wastewater samples. Heterotrophic bacteria were enumerated by HPC using 3M petrifilm as nutrient medium. A dilution series (10^{-3}–10^{-6}) of the samples were prepared by serial dilution, and a portion of 1 mL diluted sample of sludge was transfered to the prepared 3M petrifilm. 3M Petrifilm AC Plates are used for the enumeration of aerobic bacteria. The 3M™ Petrifilm™ Aerobic Count (AC) Plate is a ready-made culture medium system that contains Standard Methods nutrients, a cold-

water-soluble gelling agent and an indicator that facilitates colony enumeration. After the incubation of 48 hours at 35 °C, a red indicator dye in the plate colors the colonies. All red colonies are counted on colony counter (Stuart SC6) regardless of their size or color intensity. The preferable counting range on a 3M Petrifilm AC Plate is between 25–250 colonies. Measurements are recorded as colony forming units (cfu/mL) and multiplied by the dilution factor. All media ingredients were purchased from Era Bumi Sains Sdn Bhd (1054116-P), Selangor, Malaysia.

The equipment used for the colony counting (Stuart SC6) and bacteria incubator are illustrated in Figure 3.13 a and b, respectively. It is equipped with a digital counting tally and magnifier that makes counting convinient and easy from the colonies from on the incubated 3M petrifilm aerobic count plates.

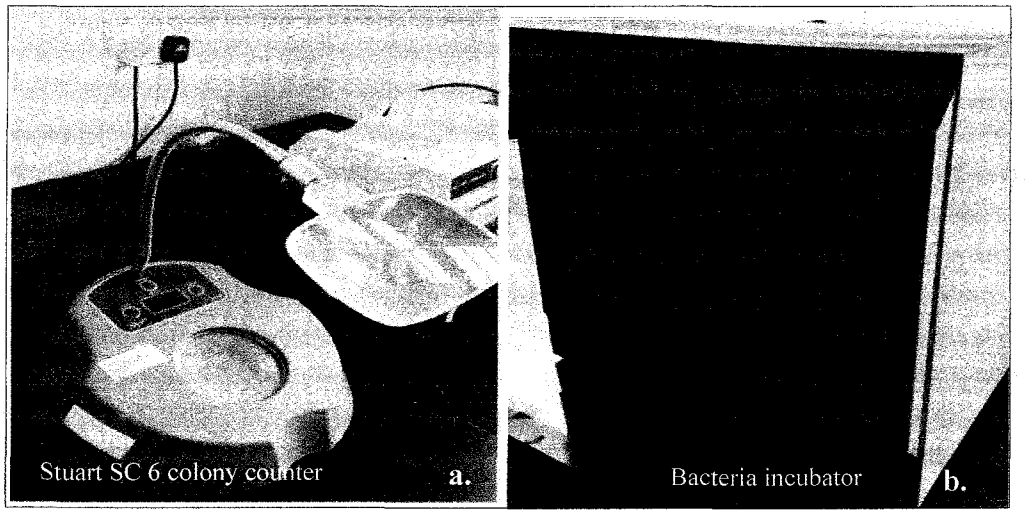


Figure 3.13: (a). Stuart SC6 counter with magnifier, and (b). Incubator

3.5.10.2 Measurements for samples of mixed liquor for microscopic analysis

To determine the microfauna from the mixed liquor, sludge samples were obtained in a two liter laboratory glass jar, which was reserved through constant aeration to homogenize the contents for the entire duration of the analyses. This practical conduct was in view to keep the initial characteristics of the sludge unaltered. Samples were analysed 30 minutes after collection. The counts of the microfauna in activated sludge sample were accomplished under an optical microscope on 400X magnification, using three replicates of 100 µL sub-samples taken at random with a 100 µL automatic micro

pipette. A sample of mixed liquor drop is deposited on a glass slide and carefully covered with a slip to avoid any mechanical stress on the microorganisms. The procedure has been outlined elsewhere [110, 280, 306]. The microscopic analysis to count protozoa and metazoa were performed consistently within 3 hours after sample collection. Five fields of view were counted in a vertical plane and the entire process was completed within 20 minutes. The optical microscope (Leica DM LB2, Japan) equipment and computer monitor used to process the images is presented in Figure 3.14.

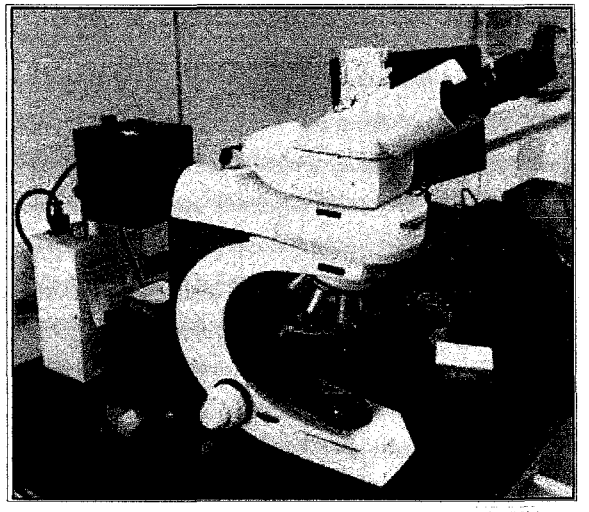


Figure 3.14: Leica DM LB2 microscope and computer

3.6 Computations of other performance and operational variables

To compute the common variables for operation, Equations 3.20 through 3.32 in Table 3.7 were used to determine the common operational parameters and other useful performance indicators in the study. These parameters include the HRT, SRT, F/M ratio, OLR, nitrification and denitrification efficiencies, specific nitrification and denitrification rates, ammonia loading rates (ALR), and the removal efficiencies. The removal efficiencies include COD, BOD_5, TSS, total nitrogen, ammonia nitrogen, total Kjeldahl nitrogen and total phosphorus.

Table 3.7: Equation used to evaluate variables for the analysis

Parameter	Equation	Reference
HRT_{ANX-C} (h)	$\frac{V_{ANX-C}}{Q_{Inf}}$ (3.20)	[84] [262]
HRT_{AER-C} (h)	$\frac{V_{AER-C}}{Q_{Inf}}$ (3.21)	
HRT_{Total} i-SGBR (h)	$\frac{V_{i-SGBR}}{Q_{Inf}}$ (3.22)	
SRT_{I-SGBR} (d)	$\frac{VX}{X_r Q_w}$ (3.23)	
F/M ratio (d^{-1})	$\frac{F}{M} = \frac{QS_O}{VX}$ (3.24)	[268]
Organic loading rate, OLR, (kg COD/m^3 d))	$OLR = \frac{QS_O}{V}$ (3.25)	[84]
Nitrification efficiency (%), η_N	$(1 - \frac{NH_3 - N_{Eff}}{NH_3 - N_{Inf}}) * 100$ (3.26)	[20, 120]
Nitrates in ANX-C (mg/L), $NO_3 - N_{ANX-C}$	$\frac{(Q_{In} * NO_3 - N_{Inf}) + (Q_{IR} * NO_3 - N_{AER-C}) + (Q_{RAS} * NO_3 - N_{Eff})}{Q_{In} + Q_{IR} + Q_{RAS}}$ (3.27)	
Denitrification efficiency (%), η_D	$\left[\frac{(NO_3 - N_{Inf_{ANX-C}} - NO_3 - N_{Eff-ANX-C})}{NO_3 - N_{Inf_{ANX-C}}}\right] * 100$ (3.28)	
Specific nitrification rate, $r_{N,}$ (mg.NH_3-N/g. VSS.d)	$\frac{Q_C(NH_3 - N_{Inf} - NH_3 - N_{Eff})}{V_{AER-C} * VSS_{AER-C}}$ (3.29)	
Specific denitrification rate, $r_{D,}$ (mg.NO_3-N/g. VSS.d)	$\frac{Q_{In}(NO_3 - N_{Inf\_ANX-C} - NO_3 - N_{Eff\_ANX-C})}{V_{ANX-C} * VSS_{ANX-C}}$ (3.30)	
Ammonia loading rate, ALR, (mg/L.d)	$\frac{Q_{In} * NH_3 - N_{Inf}}{V_{AER-C}}$ (3.31)	
Removal efficiency	$Efficiency = \frac{Coi - Cof}{Coi} * 100$ (3.32) Co_i = Initial concentration, mg/L, and Co_f = Final concentration, mg/L	[307]
$\Delta COD/\Delta NO_3$-N	Ratio = (infl. COD – effl. COD)/infl. nitrate N- effl. nitrate N (3.33)	[252]

3.7 Statistical analysis

Microsoft excel 2013 was used to record and manage all the experimental data during the period of analysis. Numerous spread sheets were created for usage to handle both single and multiple parametric analysis. This was achieved through establishing the formulae equations to automatically obtain the outputs.

3.7.1 Descriptive statistics

The descriptive statistics was explored to define basic features of the data in this research. The summaries about the sample, measures, graphics analysis were carried out to organize, and present the quantitative analysis of the data so that it can be readily understood. The important information to be extracted from a data set is the measure of central tendency and variability.

Averages and standard deviations were used to express the variations in concentrations. The analysis was based on 95 % confidence interval. The assessments were carried out to compare the system performance, and effects of different parameters operated on i-SGBR pilot study; such as effects of varying internal recycle rate on denitrification efficiency, and effects of HRT on treatment efficiencies on COD, TSS and ammonia removal. Hence, the data analysis was statistically supported to assess the parameter influence on the process performance in order to lay significant conclusions. The one-way analysis of variance (ANOVA) without replication was used to determine whether there were any significant differences between the means of two or more independent (unrelated) groups [308]. Then the one-way ANOVA analysis was followed up by the two sample t-test with unequal variance, as the statistical data analysis procedure for hypothesis testing. A value $P<0.05$ was used to define significant results [309, 310]. In the subsequent discussion of results, the significance of the statistical analysis is presented in brackets (i.e. $p<0.05$) whenever a statistical assessment was conducted to determine if a comparison was statistically significant. MATLAB 7.8.0 (R 2009a) was used to run the ANOVA tests and an ad-hoc test called multiple comparison to simultaneously test set of statistical inferences based on observed values.

In chapter 4, the time series plots and bar charts were used to present as well as interpret performance from trend of the measured concentrations for each of the parameters analyzed at steady state. The presentation of data was based on minimum, maximum and average values. The plot of these values was based on average values, while collectively the data for minimum, maximum and average values were summarized. Therefore, summary from the statistical analysis is contained within the results and discussion chapters, whereas raw data of the analysis are provided in individual Appendices.

3.8 Quality control

The long-standing definition of quality was "conformance to specification" [311]. The equipment pumps and sensors for i-SGBR were calibrated earlier before the start-up and operation. The diaphragm pumps were maintained every month through flushing and backwashing according to manufacturer's requirement manual.

Samples were well preserved in a clean glass container ahead of each analysis to be carried out. These samples were analyzed in triplicates to ensure non-bias of results. To avoid human related error, each sample was properly and carefully labeled. To further comform with quality criteria, some samples were randomly analyzed using different equipments within the laboratory, and results compared for its consistency.

Measured parameters such as COD, BOD_5, ammonia-nitrogen, nitrate-nitrogen, TKN, TN, MLSS, MLVSS and TSS were analysed in triplicate to ensure consistency of data, and was subjected to statistical validation such as averages and standard deviations. Other tools for performance measurement such as ANOVA and multiple comparison using MATLAB 7.8.0 (R 2009a) were used for validation of data.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Chapter overview

This chapter presents the results obtained from the study followed by discussion. The results are reported for the three phases according to the experimental design outlined. The phases are categorized as follows:

i. Phase I: Results for design and fabrication of i-SGBR are presented and discussed here, where volumes of the anoxic, aeration, aerobic digester and clarifier chambers were determined.

ii. Phase II: In this phase, results are presented for the operation and control parameters such as MLSS, MLVSS, SVI, pH, temperature, DO and F/M ratio and SRT. Start up and operation to evaluate the effect of HRT and IR on i-SGBR treatment performance with simultaneous aerobic digestion. Q of 45, 70 and 100 L/d were used and IR ratios of 6 and 3 were operated. Results for the performance of the i-SGBR system is also presented for the removal of the organic matter (COD, sCOD, and BOD_5), nitrogen (ammonia-nitrogen, nitrate-nitrogen, TKN, and TN), total phosphorus, and TSS. Sludge degradation was evaluated based on MLSS and MLVSS reduction in the AD-C system.

iii. Phase III: In this phase, results are presented and discussed for the operation and control parameters such as MLSS, MLVSS, SVI, pH, temperature, DO and F/M ratio and SRT, as well as the results for system performance regarding removal of the organic matter (COD, sCOD, and BOD_5), nitrogen (ammonia-nitrogen, nitrate-nitrogen, TKN and TN), total phosphorus, and TSS. Sludge degradation was evaluated based on MLSS and MLVSS reduction in AD-C

system. Results for evaluation of bio-kinetic parameters for carbon oxidation and operation of simultaneous aerobic digestion was discussed.

4.2 Results for wastewater characteristics of the study

Parameters such as COD, sCOD, BOD_5, TSS, TP, ammonia-nitrogen, nitrate-nitrogen, total nitrogen, TKN, and pH were measured in the effluent released into the balance tank of the WWTP. This wastewater was used as influent to operate i-SGBR system.The characteristics of the influent wastewater used for this study is shown in Table 4.1.

Table 4.1: Characteristics of influent wastewater

Parameter	Max.	Min.	Mean±SD
COD (mg/L)	1593	715	1223±234
COD (diluted sample) (mg/L)	1096	952	1019±28.8
sCOD	591	297	454±62.4
BOD_5 (mg/L)	851	760	794±68
TSS (mg/L)	788	360	614±107
TP (mg/L)	60.8	31.8	51.1±5.7
Ammonia-nitrogen (mg/L)	57.0	48.2	52.6±2.2
Nitrate-nitrogen (mg/L)	0.6	0.1	0.4±0.2
Total-nitrogen (mg/L)	94.7	64.7	82.9±6.6
TKN (mg/L)	94.5	64.0	82.5±6.7
pH	4.2	6.8	5.89±0.61

Influent COD ranges between 715 to 1593 mg/L, TN ranges between 64.7 to 94.7 mg/L, and the total phosphorus ranges between 31.8 to 60.8 mg/L. The corresponding average and standard deviation concentration were 1223±234 mg/L, 83±7 mg/L, and 51±6 mg/L, respectively. Conversely, this gives an average of C/N/P ratio of 100/7/4 for the wastewater. To effectively accomplish biological treatment process, influent C/N/P ratio for wastewater should normally be above 100/5/1, as corroborated from previous work according to Section 2.10.

The approximate sCOD and BOD_5 contributions were 36 % and 65 % of the COD, respectively. This corresponds to influent COD/BOD_5 ratio of 1.54, which probably indicates eminent biodegradability nature of the wastewater, and could essentially be

high in organic its content. This ratio is possible for FBI [41]. The high variability in the influent COD concentration might be due to the nature and quantity of daily production. Destruction of reject products that did not pass QA requirements could lead to high or low pH, thereby serving as potential point source to increase effluent organic loading entering the WWTP.

In this research, about 63 % of total nitrogen comes from ammonia-nitrogen. The high content of the organic-nitrogen might be due to the transportation in the sewer network and storage of the wastewater in the balance tank. Total nitrogen (TN) comprises both organic and inorganic; ammonium, nitrate and nitrite forms [82]. Nitrogen in wastewater originates from human fecal matter, and industrial waste particularly food processing waste. Influent nitrogen comprises of three main sources: approximately 60 % of ammonium; 40 % of organic nitrogen which consists of a complex mixture of organic compounds including amino acids, amino sugars, and proteins; the negligible amount of nitrate, normally less than 1 % [83].

The waste stream from the FBI production activities could be mainly due to residual ingredients from cleaning, which comprise extract of malted barley, solid milk, cocoa, sugar, butter oil, palm oil, soya oil, cocoa-nut oil, grinded coffee, minerals and vitamins (including sodium phosphate, magnesium carbonate, ascorbic acid and di calcium phosphate). The source of the wastewater was from the cleaning operations, where alkali (NaOH, 30-60 %) and acids (phosphoric acid < 30 %, vortex, acetic acid <24 %) were used. The wastewater from the toilets (urine and faeces), and organic food waste make up the wastewater.

4.3 Phase I results for design and fabrication of i-SGBR bioreactor system

The individual bioreactors constituting i-SGBR include ANX-C, AER-C, AD-C, and CLR. They were designed separately based on the input and ouput from each bioreactor. Then, integrated together as a single modular component. The rounded up dimension features for ANX-C, AER-C, AD-C, and CLR chambers; the height, diameter, area and volume are presented in Table 4.2. The working volumes for each chamber is specified

in liters (L). A portion of the chamber volumes was left as freeboard to accommodate the space required to avoid overflow due to mixing and aeration turbulences.

Table 4.2: The design dimensions of integrated bioreactor system

Chambers	Height (mm)	Diameter (mm)	Net base area (mm^2)	Lateral surface area (mm^2)	Total area (mm^2)	Working volume (L)
Influent storage	1600	350	96,211	175,9292	1,855,503	150
ANX-C	820	250	49,087	644,026	693,114	40
AER-C	435	655	287,868	1,539,145	1,827,013	125
AD-C	455	800	165,699	2,038,658	2,204,357	75
CLR	520	935	-	2,377,243	3,210,727	100

Bioreactor design calculations comprising ANX-C, AER-C, AD-C, and CLR are detailed in Appendix A1.1. Configuration of i-SGBR system is illustrated in Figure 4.1.

The ANX-C is the primary treatment chamber, with working volume and total effective area of 40 L and 693, 114 mm^2, respectively. The dimensions are 900 mm as total height (820 working height) and diameter of 250 mm. The ANX-C consist of a slender column with ring baffles, which was constructed to enhance the sludge retention and avoid possible washout of biomass.

The AER-C has a volume and total effective area of 125 L and 1,827,013 mm^2, respectively. The dimensions are the total height of 800 mm (working height 435 mm) and diameter of 405 mm. The AD-C has a volume and total effective area of 75 L and 2,204,357 mm^2, respectively. The dimensions are the total height of 800 mm (working height of 455 mm) and diameter of 145 mm. The CLR has a volume and total effective area of 100 L and 3,210,727 mm^2, respectively.

The integrated bioreactor units have segregated chamber compartments that can easily be assembled, inspected and maintained. ANX-C is fitted differently with a baffle to control detention time of biomass and washout. AER-C and AD-C are fixed together, while they collectively rest inside CLR to settle sludge as by-product of the biological process.

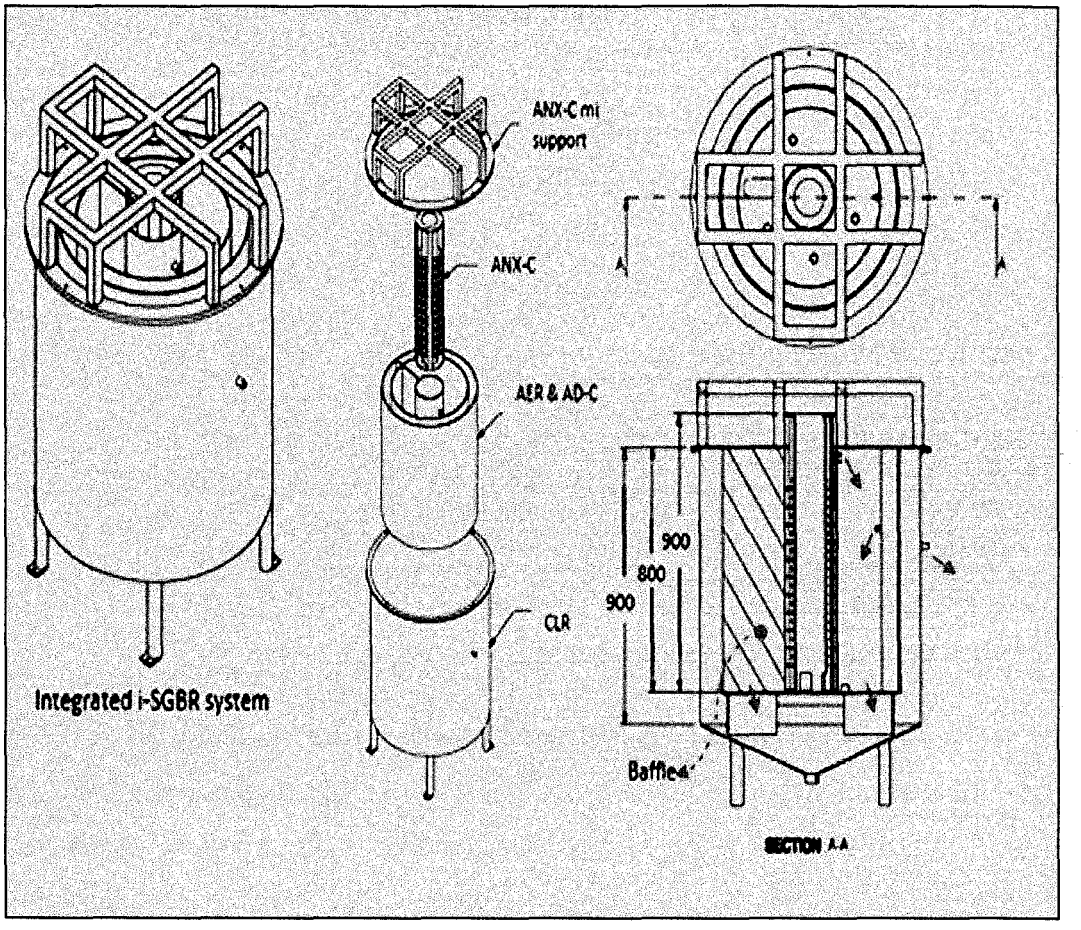


Figure 4.1: The configuration of integrated bioreactor system

The dimensions are total height 1000 mm (including the underflow bottom conical shape, 100 mm deep) and working height of 520 mm and diameter of 135 mm. Schematic diagram of i-SGBR used for the pilot study is illustrated in Figure 4.2.

The fabrication of i-SGBR was done to include a feed tank with a capacity of 150 L, control panel, air operated diaphragm pumps, ANX-C, AER-C, and AD-C, and CLR chambers. The fabrication and supply of i-SGBR system were made by Solution Engineering (SOLTEQ) Sdn. Bhd., Malaysia. Material for i-SGBR was made using 5 mm thick stainless steel. The total effective volume of i-SGBR system is 340 L, and space footprint; 1300 mm height, 1060 mm width, and 1760 mm depth fitted with a frame to provide support.

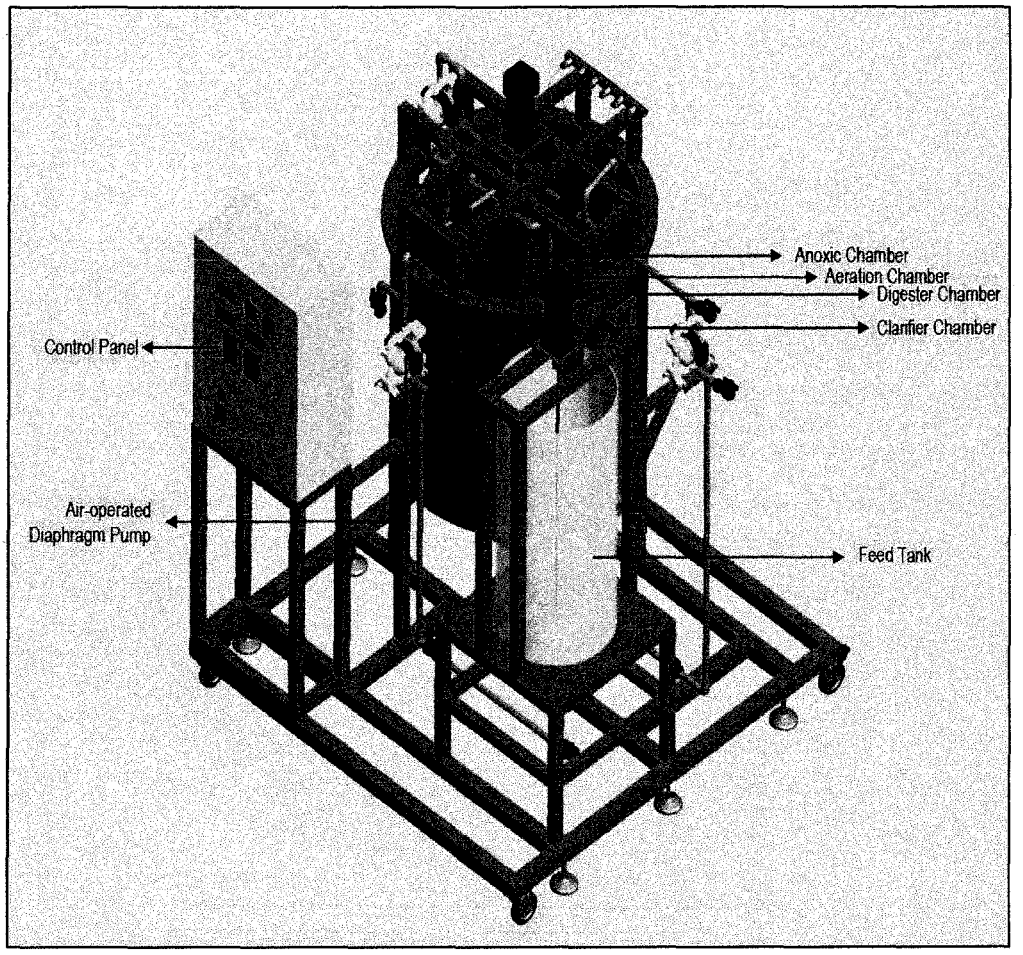


Figure 4.2: The schematic diagram of i-SGBR process

4.4 Phase II results for system operation and control parameters

This section presents the results for operation and control parameters of i-SGBR system. This includes the actual parameters operated for MLSS, MLVSS, SVI, pH, DO, temperature, HRT, OLR, F/M, SRT, IRQ, and RAS flow.

Operation in phase II was carried out between days 1-162 using three flow rates of 45, 70 and 100 L/d. Simultaneous aerobic digestion was monitored in a separate chamber, where RAS was fed into it and daily comparison was made between digested mass and wasted mass. The overall goal was to achieve steady state operation for the phase. In phase II, four stages were operated which include phase II-A through phase II-D.

4.4.1 Phase II results for MLSS, MLVSS, and SVI

This section presents the results monitored for MLSS, MLVSS, and SVI for phase II. ANX-C was monitored for MLSS and MLVSS to evaluate the parameters such as specific denitrification rates. AER-C was monitored for MLSS, MLVSS, and SVI to evaluate parameters for SRT control, F/M ratio, and sludge settling properties. CLR was monitored for MLSS and MLVSS to evaluate the RAS recharge to the AER-C, sludge wasting, and assessment of the mass balance between the mass of digested sludge in AD-C and mass of daily wasting from CLR underflow. RAS MLSS concentration was monitored from laboratory and validated with calculated values evaluated from AER-C MLSS and SVI according to Section 3.5.9.

4.4.1.1 Phase II for anoxic chamber MLSS and MLVSS concentration

The changes in the biomass concentration for ANX-C at different OLR and HRT from phase II-A to phase II-D are shown in Figure 4.3 and ANX-C OLR is shown in Figure 4.4.

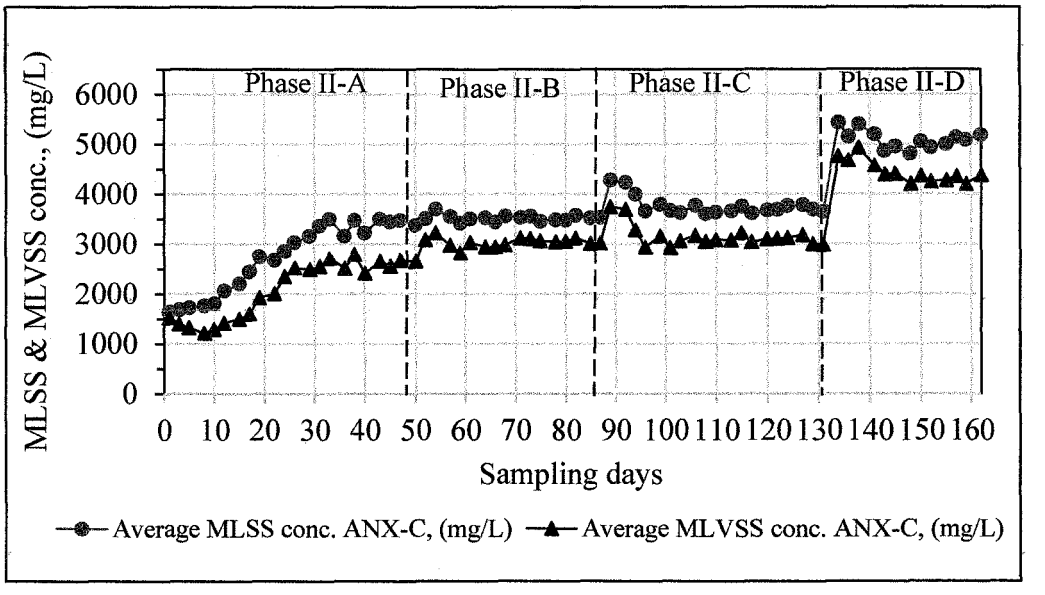


Figure 4.3: Anoxic chamber Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids Conc. vs Sampling Days

In phase II-A, ANX-C was operated with OLR having average and standard deviation values of 1.34±0.68 kg COD/m^3.d. Corresponding ANX-C HRT was 21.0 hours. The profile of the ANX-C OLR throughout phase II-A to phase II-D is shown is Figure 4.4.

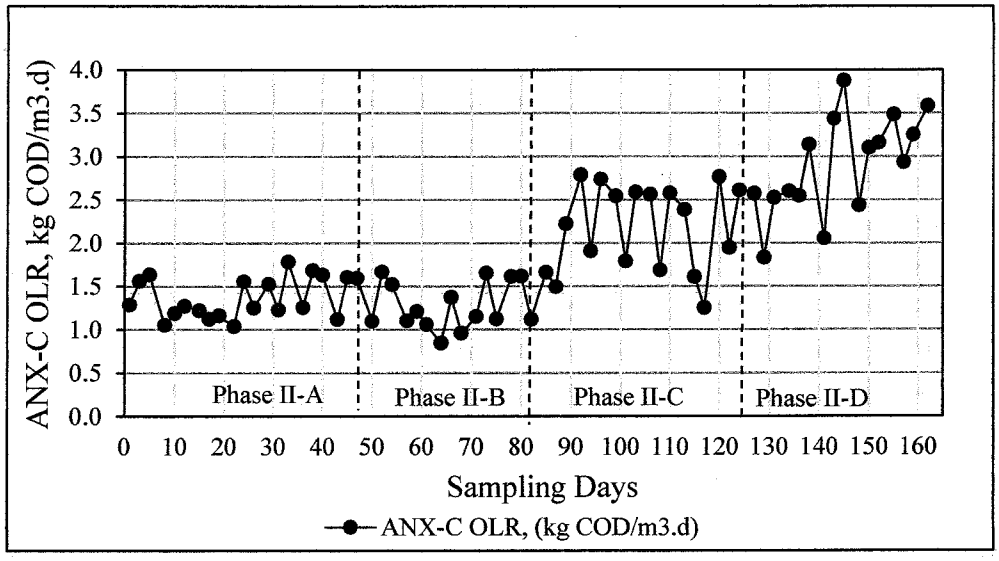


Figure 4.4: Organic Loading Rate for Anoxic chamber vs Sampling Days

ANX-C chamber was the first reaction chamber to receive the influent substrate, biomass concentration from RAS and IR. At the start up, RAS ratio was set at 0.8 corresponding to RAS flow of 35 L/d and IR ratio for 3 corresponding to IR flow of 135 L/d. Operation in phase II-A was done to build up the biomass and stabilize the system. Thus, the biomass used for the inoculation needs time to acclimatize to the new environment. Refer to Figure 4.3. Hence, operation between day 1 to day 50 was to ensure the system was steady prior to subsequent operations. It was observed that the reactor had low biomass concentrations in ANX-C in the beginning. Biomass was observed to gradually build up in the reactor system and accumulate without wasting, where on day 26, MLSS and MLVSS concentrations in ANX-C were found to be 3026 mg/L and 2526 mg/L. On day 33, IR ratio was increased from 6 to 3, and IR flow of 270 L/d was operated. It could be observed from Figure 4.3 that there was a rise in ANX-C MLSS and MLVSS concentration up to day 33. From day 43 to day 50, steady state in ANX-C MLSS and MLVSS concentration were observed. At the steady state, average and standard deviation values for ANX-C MLSS and MLVSS were found to

be 3449±54 mg/L and 2672±93, respectively. This gives ANX-C MLVSS/MLSS ratio of 0.77. The observed increase in biomass concentration in ANX-C could be due to increase in the IR flow from 135 L/d to 270 L/d, where more biomass was supplied before it was washed off and eventually stabilizes.

In phase II-B operation, OLR for ANX-C was the same operated as phase II-A operation. RAS flow of 35 L/d and IR flow of 270 were maintained as the latest from the previous phase II-A operation. Aerobic digestion monitoring started on day 50, however, there was significant visible effect in the ANX-C solids trend observed, which might be due to the operation of an aerobic digester on day 54, where ANX-C MLSS and MLVSS peaks to concentrations of 3697 mg/L and 3227 mg/L, respectively. For the subsequent days for phase II-B operation, ANX-C MLSS and MLVSS concentrations remained relatively stable from day 61 to day 87, where average and standard deviation values were obtained as 3454±68 mg/L.

In phase II-C, OLR for the operation was increased for ANX-C OLR from 1.34±0.68 kg COD/m^3.d to 2.26±0.46 kg COD/m^3.d, and decrease in HRT from 21 hours to 13.7 hours from phase II-B , where there was obvious increase in ANX-C MLSS and MLVSS concentration observed on day 89 as 4277 mg/L and 3743 mg/L, respectively, and gradually stabilizes on day 101 to 3665 mg/L and 2919 mg/L, respectively. The rise could be due to increase in the ANX-C OLR and decrease in HRT from phase II-B. Two IR flow regimes of 420 L/d and 210 L/d were operated, with IR flow of 420 L/d between day 89 to day 110, and IR flow of 210 L/d between days 113 to 131. However, no noticeable effect could be observed in distortion for the trend of biomass concentration for the ANX-C MLSS after the steady state from day 101. The average and standard deviations values for ANX-C MLSS and MLVSS at steady state were observed to be 3691±73 mg/L and 3061±103 mg/L, respectively. This gives corresponding ANX-C MLVSS/MLSS ratio of 0.82.

In phase II-D, the operation was carried out with further increase of ANX-C OLR, which implies reduced HRT from phase II-C. Average and standard deviation OLR values for the ANX-C was 3.05±0.52 kg COD/m^3.d. ANX-C HRT operated for phase-II-D was of 9.6 hours. It could be seen from Figure 4.3 that, there was a surge

observed in ANX-C MLSS and MLVSS concentration values on day 134 to 5,427 and 4,744 mg/L when ANX-C OLR was increased on day 131. Thus, ANX-C MLSS and MLVSS stabilized on day 143 as can be seen from Figure 4.3 to MLSS and MLVSS concentration with values of 4,863 and 4,393 mg/L, respectively. Steady state was observed between day 143 to day 162, where ANX-C MLSS and MLVSS concentration were 4,918±92 and 4,322±93 mg/L, respectively. This gives ANX-C MLVSS/MLVSS ratio of 0.89.

4.4.1.2 Phase II results for aeration chamber MLSS, MLVSS, and SVI profile

Changes in biomass trend for AER-C MLSS and MLVSS is shown in Figure 4.5, the profile for ANX-C OLR throughout phase II-A to phase II-D is shown is Figure 4.6, and profile for SVI monitored in the AER-C is shown in Figure 4.7.

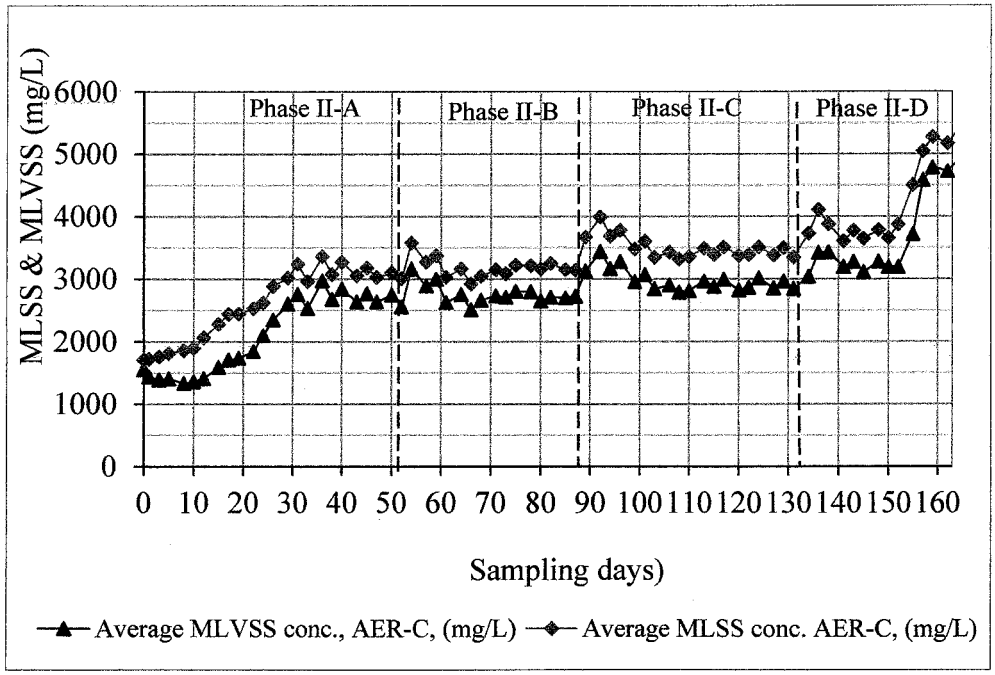


Figure 4.5: Aeration chamber Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids Conc. vs Sampling Days

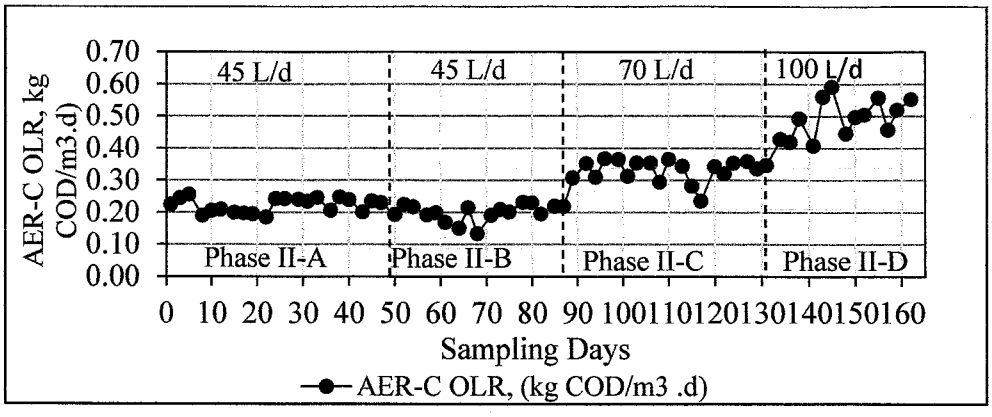


Figure 4.6: Organic Loading Rate for Aeration chamber vs Sampling Days

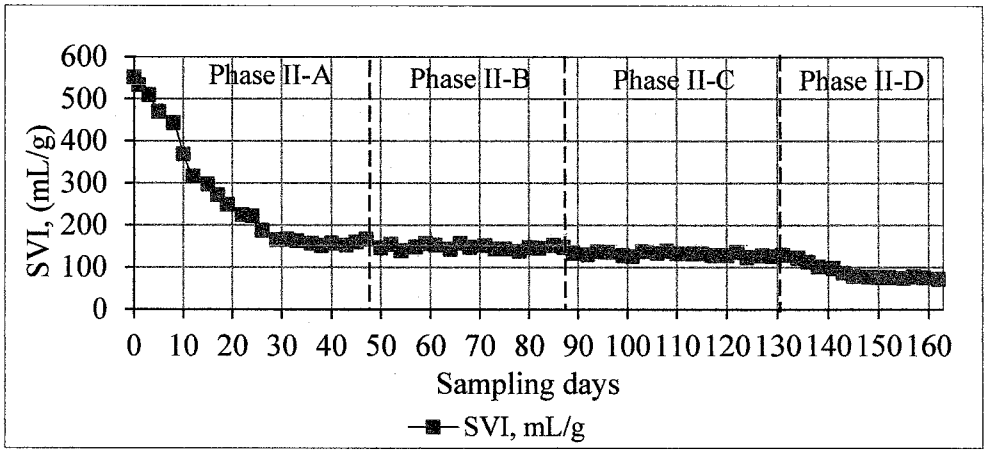


Figure 4.7: Sludge Volume Index profile vs Sampling Days

In phase II-A, AER-C was operated with OLR having average and standard deviation values of 0.21±0.03 kg COD/m^3.d, which corresponds to AER-C HRT value of 66.7 hours. This is shown in Figure 4.6. The operation between day 1 to day 50 was to stabilize the system for subsequent operations. Biomass was observed to gradually build up, where the AER-C MLSS and MLVSS concentrations accumulated without wasting to the value of 3020 mg/L and 2603 mg/L on day 29 as shown in Figure 4.5. Sludge settling between the start-up period to day 24 was observed to be poor, where the value of 222 mL/g was observed on day 24 as shown in Figure 4.7. SVI improved on day 29 with the observed value of 165 mL/g. On day 33, IR ratio of 3 (IR flow of 135 L/d) was operated. For AER-C steady state was observed between day 43 to day 50 with AER-C MLSS and MLVSS concentrations found to be 3113±102 mg/L and 2711±103 mg/L, respectively. This gives AER-C MLVSS/MLSS ratio value of 0.87. There was no observed increase in biomass concentration in AER-C on day 33 to day,

contrary to the observed rise in the ANX-C MLSS and MLVSS concentrations, which could be due to increase in the IR flow from 135 to 270 L/d, in which more biomass was supplied before it was washed off and eventually stabilizes. Instead, the AER-C MLSS and MLVSS concentration were observed to slightly declined to AER-C MLSS and MLVSS values of 2,963 and 2,533 mg/L, respectively. This may be due to a temporary sludge withdrawal from the AER-C. Adequate DO supply with sufficient mixing could influence better SVI values observed between day 33 to day 50 as average and standard deviation 157±6.6 mL/g were observed. Biomass wasting was carried out between day 33 to day 50, with average and standard deviation values for the wasting recorded as 1.54±0.05 L/d. Biomass wasting was done to release probable accumulation of inorganic and inert matter.

In phase II-B operation, OLR was increased and HRT was decreased for AER-C. The equivalent OLR for AER-C were the same operated during previous phase II-A. RAS flow of 35 L/d and IR flow of 270 L/d were maintained in this phase. Aerobic digestion monitoring started on day 50. Although, on day 54, it could be observed from Figure 4.5 that there were more solids as AER-C MLSS concentration of 3580 mg/L as compared with day 52, where observed AER-C MLSS concentration of 3010 mg/L was obtained. On day 66, AER-C MLSS gradually recedes to AER-C MLSS value of 2923 mg/L. Between day 66 to day 87, steady state was observed for AER-C with average and standard deviation values of AER-C MLSS and MLVSS concentration of 3,191±143 and 2,770±151 mg/L, respectively. This gives AER-C MLVSS/MLSS ratio value of 0.87. SVI for the whole of phase II-B as shown in Figure 4.7 was observed to be steady with average and standard deviation values of 148±5.4 mL/g.

In phase II-C, AER-C OLR for the operation was increased from 1.34±0.68 kg COD/m^3.d to 2.26±0.46 kg COD/m^3.d as shown in Figure 4.6, where there was an observable increase in AER-C MLSS and MLVSS concentrations observed on day 89 to the concentration of 3,670 and 3,127 mg/L. These values gradually stabilize on day 103 to AER-C MLVSS and MLSS average and standard deviation concentration values of 3,550 and 2,843 mg/L, respectively. This rise could be due to increase in the OLR and decrease in HRT from 21 hours to 13.7 hours from phase II-B. In spite of two IR ratio regimes of 420 and 210 L/d operated between days 89 to 110, and between

days 113 to 131, there was no noticeable effect observed in the trend of biomass concentration for the AER-C MLSS and MLVSS from day 103 onwards to day 162 when steady state was operated. The average and standard deviations values for AER-C MLSS and MLVSS at steady state were observed to be 3,479±136 and 2,958±135 mg/L. This gives MLVSS/MLSS ratio of 0.85. It was also observed that SVI was stable in phase II-C as shown in Figure 4.7, with further decrease in the SVI value from phase II-B to average and standard deviation values of 132±4.7 mL/g.

The operation in phase II-D was done with an additional increase in AER-C OLR, which suggests reduced HRT from phase II-C (refer to Figure 4.6). Average and standard deviation AER-C OLR values were 0.50±0.06 kg COD/m^3.d. This is equivalent to AER-C HRT of 30 hours. A rise in AER-C MLSS and MLVSS values on day 134 can be observed according to Figure 4.5 when AER-C OLR was increased on day 131. Consequently, AER-C MLSS normalizes on day 141 with AER-C MLSS value of 3,607 mg/L. Between day 141 to day 152, steady state was observed for AER-C, with AER-C MLSS and MLVSS concentrations of 3,745±96 and 3,212±69 mg/L, respectively. This gives AER-C MLVSS/MLVSS concentration of 0.85. SVI values were also observed to exist in steady state between day 145 to day 162 in phase II-D, where average and standard deviation values of 92±17 mL/g were obtained for SVI. Refer to Figure 4.7. Biomass was build up in the AER-C from MLSS and MLVSS concentration of 3,870 and 3,197 mg/L, respectively, on day 152 to MLSS and MLVSS concentrations of 5,170 and 4,737 mg/L, respectively on day 162. The reason was readiness for phase III operation.

4.4.1.3 Phase II results for the relationship between measured and predicted RAS concentration

In this section, MLSS concentration for RAS (X_r) observed was compared with predicted MLSS measured from AER-C MLSS and SVI according to Equation 3.18 and Equation 3.19 for the purpose of validation.

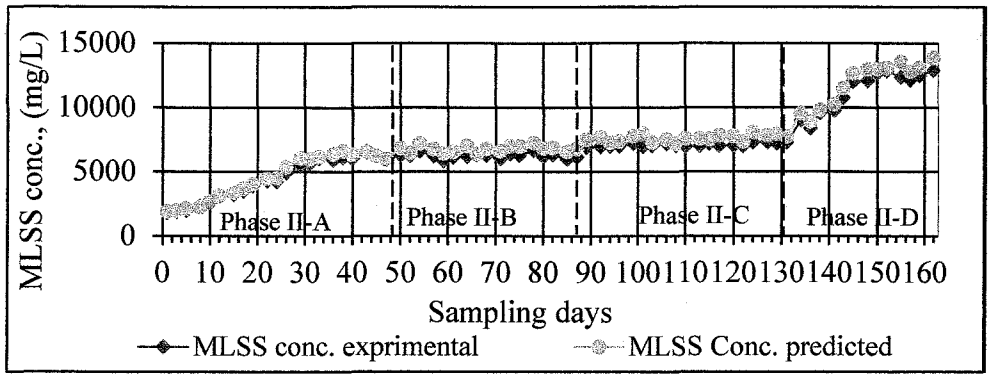


Figure 4.8: Experimental and predicted MLSS concentration vs Sample. Days

ANOVA analysis was carried out to ascertain the difference of the means between the observed and predicted MLSS concentration. At 95 % confidence interval, ($p>0.05$) there was no any significant difference observed (Table 4.3).

Table 4.3: Statistical Analysis (ANOVA) on experimental and predicted RAS concentration for phase II

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MLSS experimental	70	476724.2	6810.3	6858066
MLSS predicted	70	459739.2	6567.7	5239851

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2060630.5	1	2060630.5	0.34	0.56	3.91
Within Groups	834756274.4	138	6048958.5			
Total	836816904.9	139				

4.4.2 Phase II results for aerobic digestion and mass balance

Operating conditions of i-SGBR AD-C system has been previously described in Section 3.4.2.1. However, the AD-C was operated as batch digester system, where it was fed once daily and decanted once daily based on 10 days SRT. The volume of 7.5 L RAS was fed daily and 7.5 L was displaced daily. The objective of operating the AD-C unit was to simultaneously operate i-SGBR system by maintaining minimal sludge wastage. Therefore, in order to get rid of daily excess wastage through endogenous metabolism

in the AD-C. The approach was to return feed RAS to the AD-C so that the system will operate on little or no sludge wastage. The degradation efficiency of the AD-C was evaluated, and the mass of digested sludge from the EAD was compared with the mass of the wasting rate. The solids balance was carried out in terms of MLSS because sludge wasting is based on MLSS. Volatile solids reduction was also determined based on the aerobic digester efficiency. The detailed summary of data for solids determination in all the experiments is contained in (Table C1.1 in Appendix C).

Figure 4.9 through Figure 4.16 show the daily sequence for the variation in the levels of IAD and EAD MLSS and MLVSS concentration (mg/L), IAD and EAD mass of MLSS and MLVSS (mg/d), accumulated or degraded mass of MLSS and MLVSS in AD-C, and MLSS and MLVSS mass balance within the AD-C boundary based on Equation 3.1 through Equation 3.6 and Figure 3.7 in Section 3.4.2.1. Aerobic digestion was carried out in phase II-B through phase II-D. There was no effort made to control the daily IAD raw sludge to a particular concentration, however, it was not unexpected that there may be considerable changes in daily solids concentrations.

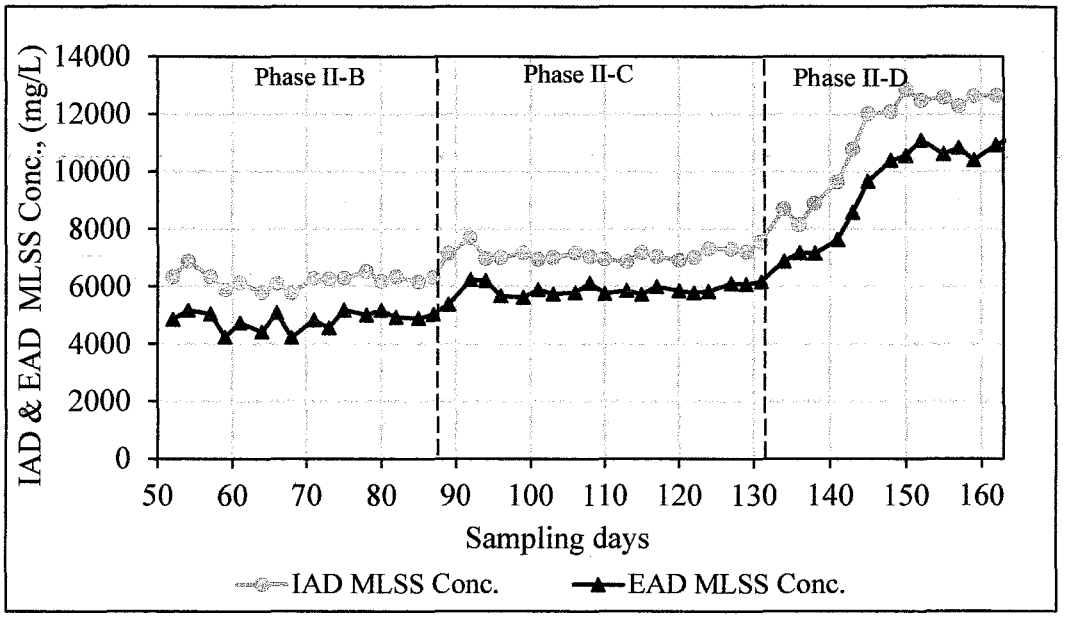


Figure 4.9: Influent and Effluent Aerobic Digester Mixed Liquor Suspended Solids Conc. vs Sampling Days

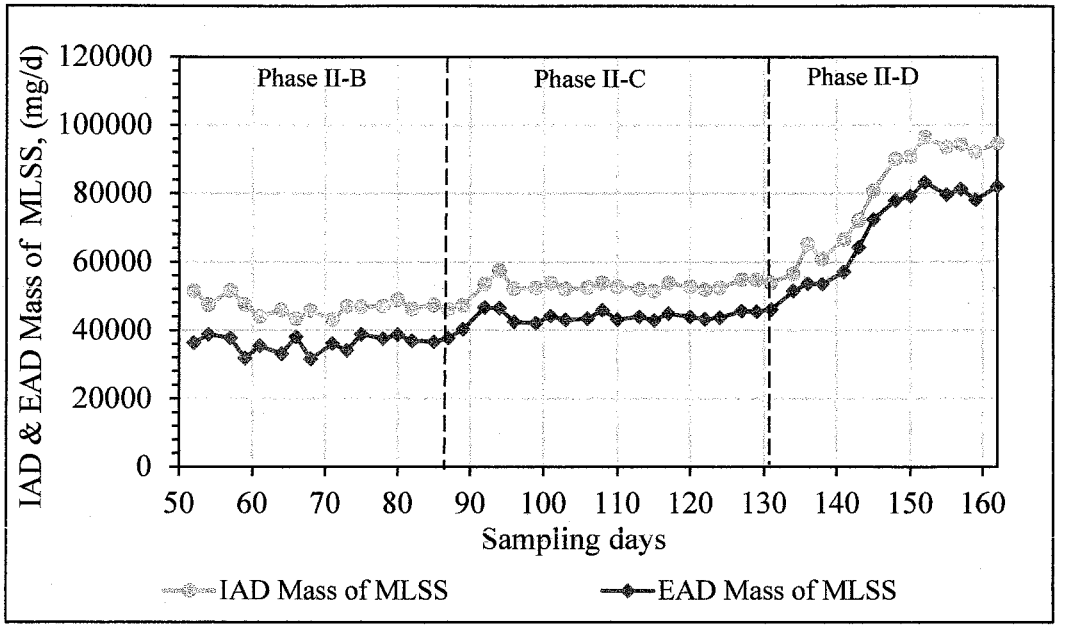


Figure 4.10: Influent and Efluent mass of Aerobic Digester Mixed Liquor Suspended Solids vs Sampling Days

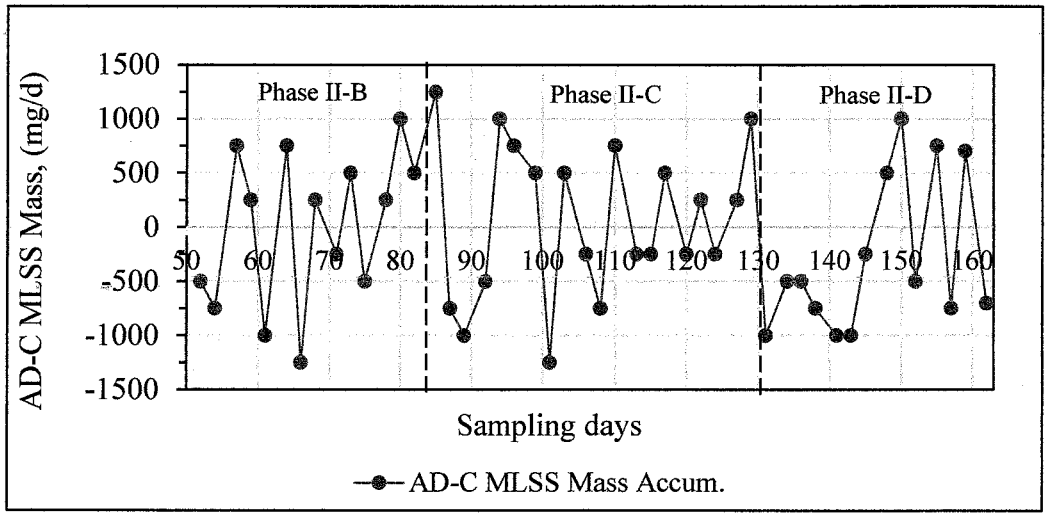


Figure 4.11: Aerobic Digester Mass Accumulation for Mixed Liquor Suspended Solids vs Sampling Days

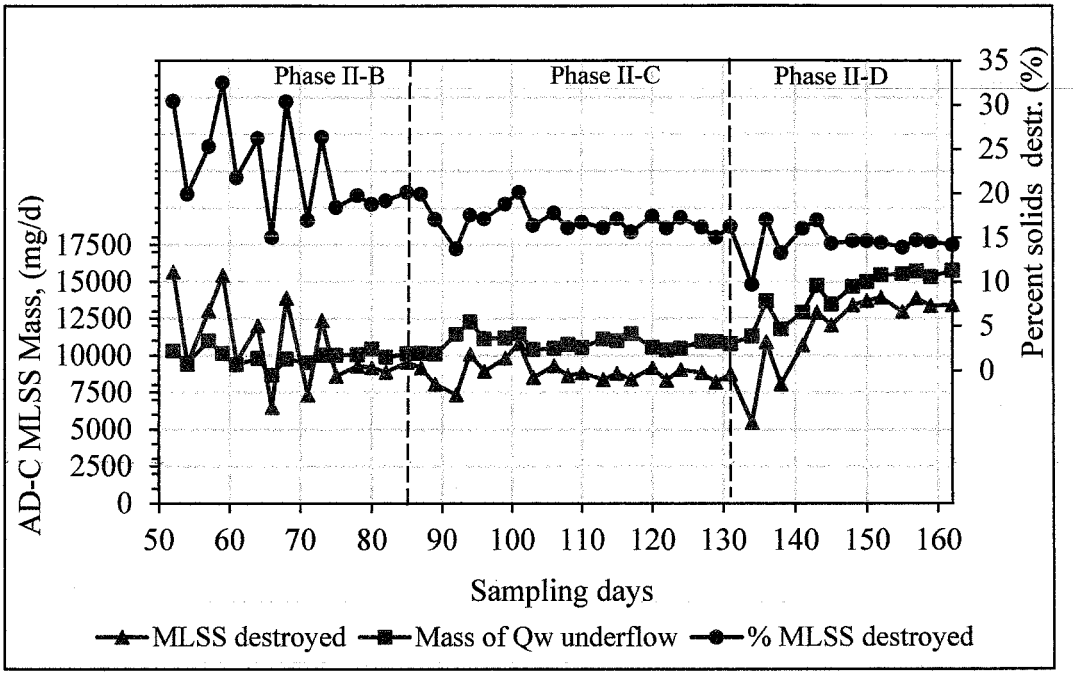


Figure 4.12: Aerobic Digester Mixed Liquor Suspended Solids Mass balance and Degadation Efficiency vs Sampling Days

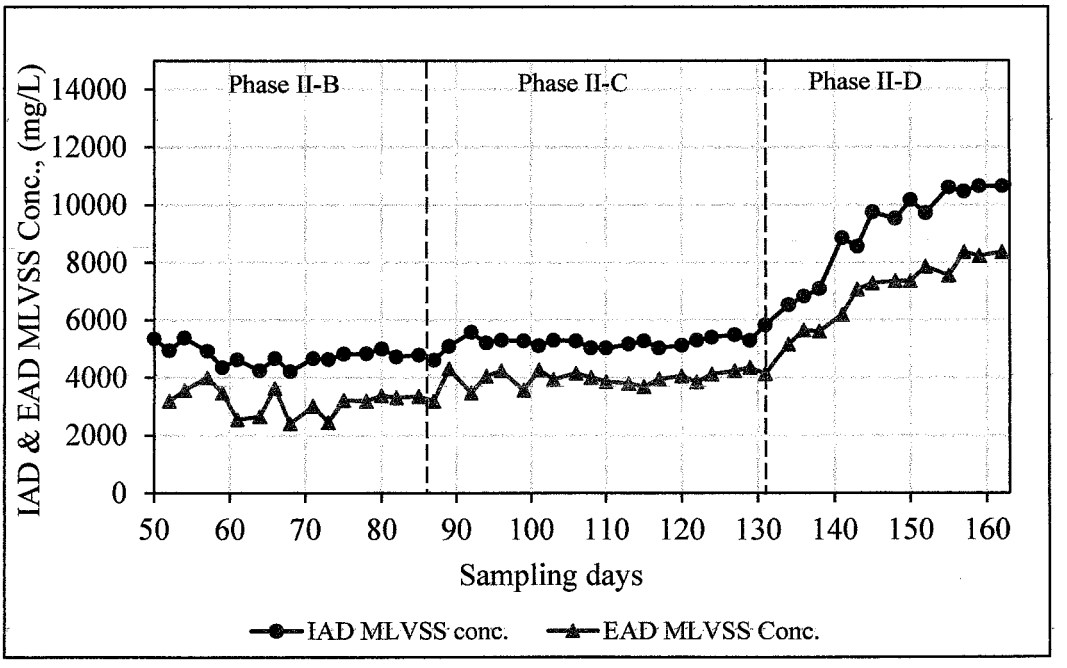


Figure 4.13: Influent and Effluent Aerobic Digester Mixed Liquor Volatile Suspended Solids Conc. vs Sampling Days

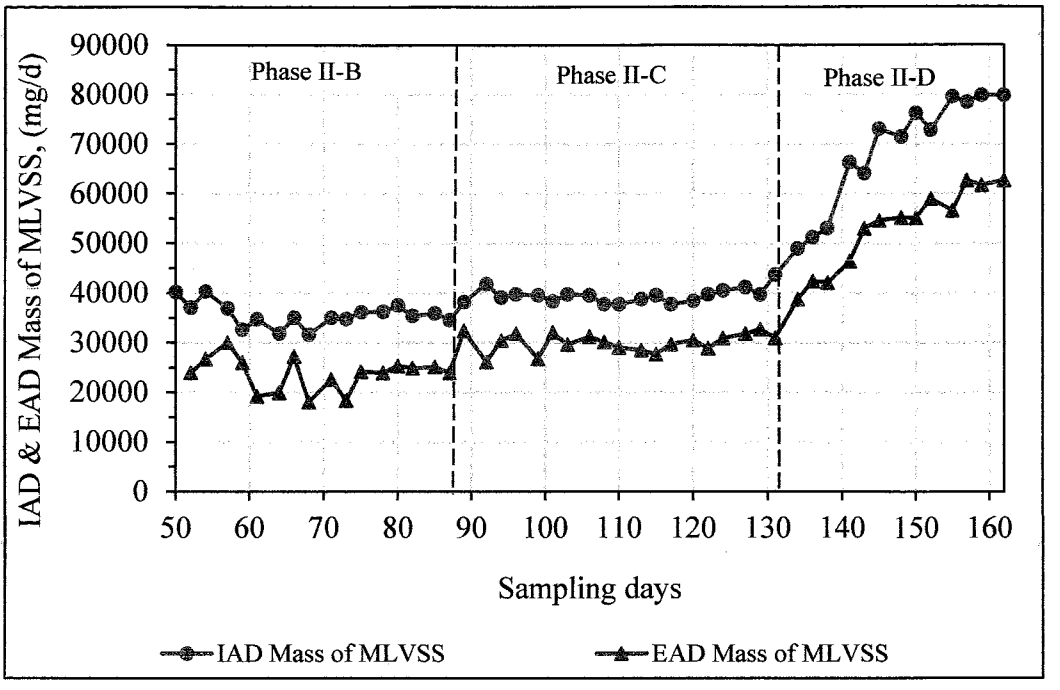


Figure 4.14: Influent and Effluent mass of Aerobic Digester Mixed Liquor Volatile Suspended Solids vs Sampling Days

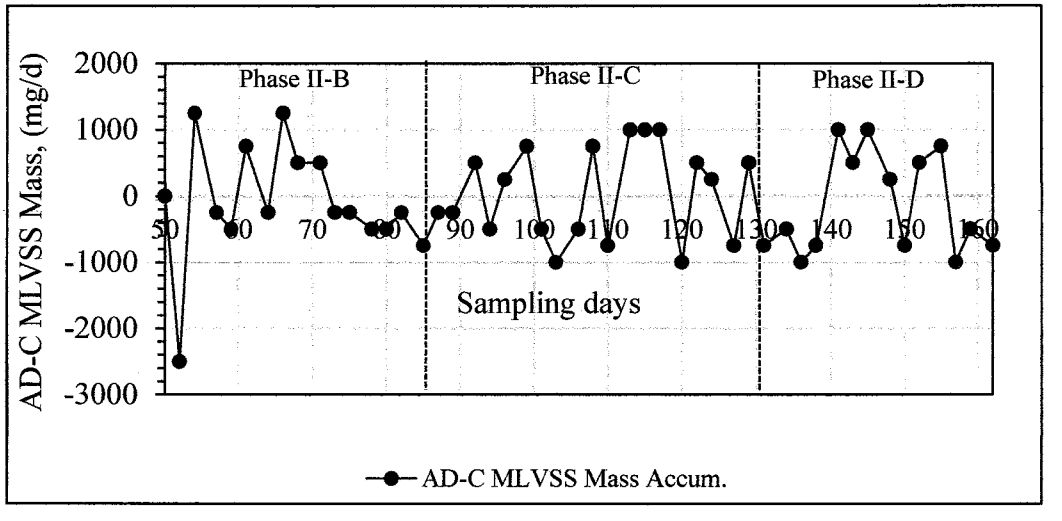


Figure 4.15: Aerobic Digester Mass Accumulation for Mixed Liquor Volatile Suspended Solids vs Sampling Days

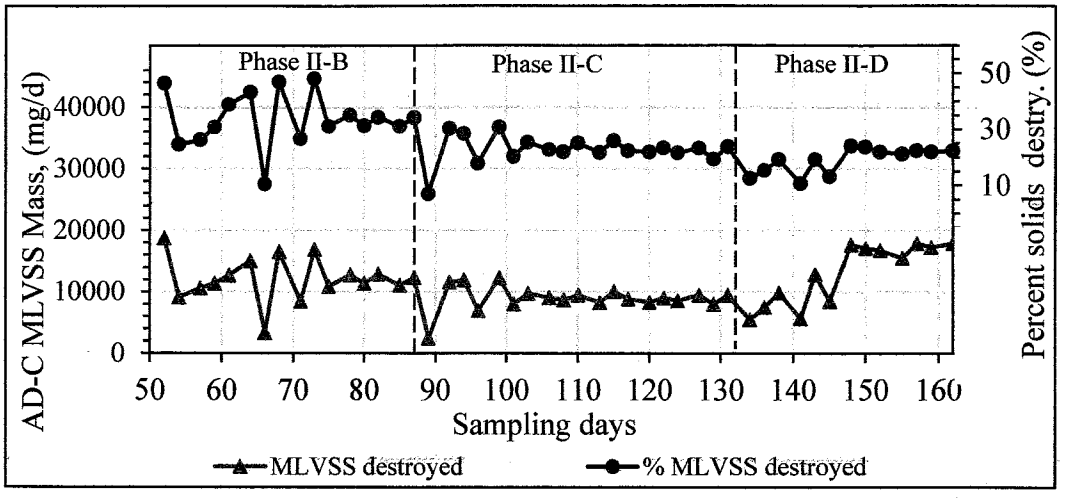


Figure 4.16: Aerobic Digester Mass balance based on Mixed Liquor Volatile Suspended Solids vs Sampling Days

The operation of the AD-C commenced in phase II-B. AD-C was seeded on day 49 with sludge from nearby sludge pit illustrated in Figure 3.8. After the initial start-up of the AD-C as shown in Figure 4.10 and Figure 4.14, there was an observed daily variation in MLSS and MLVSS profile between days 50 to day 73 in IAD and EAD MLSS, and IAD and EAD MLVSS, respectively. It was observed that IAD on day 73 had MLSS concentration of 6257 mg/L, while the EAD on day 73 with EAD MLSS concentration of 4553 mg/L. Between day 75 to day 87, steady state was observed in EAD MLSS and MLVSS concentrations, where observed average and standard deviations values for IAD and EAD MLSS, and IAD and EAD MLVSS values were observed to be (6299±133 and 5037±116 mg/L), and (4798±131 and 3278±85 mg/L), respectively. This gives equivalent IAD and EAD MLVSS and MLSS ratio of 0.76 and 0.65, respectively. The low MLVSS/MLSS ratio in the effluent could suggest that the process may have under gone endogenous metabolism, where more MLVSS were degraded than MLSS.

In phase II C, there was an observed increase in IAD MLSS and MLVSS concentration which peaks on day 92 with values of 7,680 and 5580 mg/L, respectively. Refer to Figure 4.10 and Figure 4.14. The rise in concentration could be due to increase in influent OLR from 1.34±0.68 kg COD/m^3.d in phase II-B to 2.26±0.46 kg COD/m^3.d in phase II-C. Steady state was observed between day 103 to day 131, where average

values and standard deviation for IAD and EAD MLSS, and IAD and EAD MLVSS were observed as (7,113±190 and 5,267±199 mg/L), and (5,990±305 and 4,019±187 mg/L), respectively. This gives IAD and EAD MLVSS/MLSS ratios of 0.74 and 0.69. Similar reason can be assumed for the decrease in the MLVSS and MLSS ratio from the EAD as in phase II-B.

Phase II-D was operated between day 134 to day 162, where the IAD and EAD MLVSS and MLSS concentration were observed to steadily increase until day 145 (see Figure 4.9 and Figue 4.13). Between day 145 to day 152, steady state was observed, where the average value and standard deviation for IAD and EAD MLSS, and IAD and EAD MLVSS were (12,517±272 and 10,049±604 mg/L), and (9,952±388 and 7,446±504 mg/L), respectively. This gives IAD MLVSS/MLSS and EAD MLVSS/MLSS ratios of 0.80 and 0.74, respectively. EAD ratio was observed to be lower than the IAD. This could be attributed to endogenous decay process in the AD-C. Between day 155 to day 162, the observed IAD MLSS and MLVSS concentration continued to be comparatively steady, when biomass was built up in the AER-C in readiness for the phase II operation. The underflow IAD MLSS and MLVSS concentration remained relatively stable, with average value and standard deviation of 12,526±173 and 10,585±90 mg/L, respectively. The IAD MLVSS/MLSS gives ratio of 0.84. Corresponding EAD MLSS and MLVSS concentration were observed as 10,685±236 and 8,123±390 mg/L, respectively. Ths gives a ratio of 0.76.

Figure 4.12 and Figure 4.16 show the AD-C mass balance for MLSS and MLVSS, where solids destroyed (MLSS and MLVSS) and percent solids destroyed are presented. The EAD MLSS digested solids (MLSS, mg/d) was compared with underflow mass (mg/d) (see Figure 4.12). The difference between EAD digested solids (mass of MLSS) and the wasting rate (mass of solids for IAD) were compared for SRT control. AD-C performance was evaluated accordingly (Table C1.1 in Appendix C). Wasting was carried out based on MLSS, where normally inert materials were assumed to be considered. Mass balance between AD-C boundary was evaluated according to MLSS and MLVSS mass for IAD, EAD and AD-C accumulations at various stages of steady the state periods during respective OLR operated.

Aerobic digestion commenced in phase II-B operation. It can be observed that between day 52 to day 73 during monitoring of AD-C for solids destruction. The trend has shown a slow and staggered performance of solids destruction (MLSS and MLVSS) at the begining (see Figure 4.12 and Figure 4.16). EAD mass of MLSS and MLVSS on day 52 were observed to be 36, 350 mg/d and 23, 950 mg/d with equivalent IAD MLSS and MLVSS mass of 47,425 mg/d and 37,050 mg/d, respectively. Progressive stabilization was observed from day 75, when EAD AD-C mass (MLSS and MLVSS) were observed to be steady between day 75 to day 87, with average values and standard deviation of 37, 754±892 mg/d and 24, 583±637 mg/d. The equivalent mass of IAD MLSS and MLVSS were 47, 163±1003 and 36, 021±930 mg/d. This gives degraded MLSS and MLVSS mass as 9,117±325 mg/d and 11,854±895 mg/d, respectively. Consequently, it can be observed that 19 % and 32 % of MLSS and MLVSS degradation percentages were achieved. Thus, MLSS and MLVSS accumulation between day 75 to day 87 of the steady state were 292 and -417 for MLSS and MLVSS (see Figure 4.11 and Figure 4.15), respectively. Accumulation profiles for MLSS and MLVSS are shown in Figure 4.11 and Figure 4.15, respectively. The mass of underflow during the steady period between day 75 to day 87 was observed with average value and standard deviation of 10, 113±189 mg/d. No sludge was wasted during phase II-B operation. Instead, the solids (MLSS and MLVSS) were possibly digested in the AD-C.

Phase II-C was operated between day 89 to day 101, the solids mass destruction (MLSS and MLVSS) from EAD were observed inconsistent as shown in Figure 4.12 and Figure 4.16, until between day 103 to day 131 when operation was observed to be steady with EAD stable solids pattern. As can visually be inspected from Figure 4.12, the median of the underflow wasting rate has moderately stabilized during each OLR at steady state operation. Between day 103 to day 131, average values and standard deviation for EAD solids (MLSS and MLVSS) destroyed and percentage destroyed were observed as 44,275±1191 mg/d and 16 %, and 144±1403 mg/d and 22 %, respectively. Observed mass of IAD MLSS and MLVSS were observed to be 53,006±1090 mg/d and 39,148±1101 mg/d, respectively. Accumulation of MLSS and MLVSS were both observed as 19 mg/d. Observed underflow wasting rate based on MLSS was 10,763±323 mg/d. Degraded MLSS and MLVSS were observed to be 8,712±329 mg/d and 8, 985±626 mg/d, respectively.

In phase II-D, OLR was increased on day 131, and fluctuation was observed between day 134 to day 145 from EAD solids destroyed (MLSS and MLVSS) (see Figure 4.12 and Figure 4.16). Between day 148 to day 162, steady state for EAD solids mass degradation (MLSS and MLVSS) was observed. The observed average values and standard deviation for EAD solid mass destruction and percent degradation for MLSS and MLVSS were 13,238±519 mg/d and 14 %, and 17,100±1166 mg/d and 20 %, respectively. Corresponding IAD, EAD, and accumulation for the mass of MLSS were 93,631±1179 mg/d, 80,394±2140 mg/d, and no observed accumulation of MLSS. For the MLVSS, average value and standard deviation for IAD, EAD and accumulation were observed as 77,644±3264 mg/d, 60,919±2931 mg/d and -375 mg/d, respectively. The underflow mass of MLSS was observed as 15,595±199 mg/d. MLVSS percentage degradation of 20 % was achieved in phase II-D.

A decline was observed in the AD-C solids reduction efficiency, which could be due to successive increase in concentration of IAD from phase II-B through phase II-D. Hence, this could probably be due to solids build up to likely influence the AD-C performance. The color changes and odor during the operation of the aerobic digestion were monitored. Sludge for the IAD as raw sample was observed to be brownish in color, while the digested sludge was observed as light brown. There was no observed detectable and perceptible odor in the AD-C. The continuous aeration and exposure of AD-C to open environment may play a role to control odor.

Overall summary for the steady state of mixed liquor suspended solids, mixed liqour volatile suspended solids and corresponding degradation performance during phase II is shown in Figure 4.17.

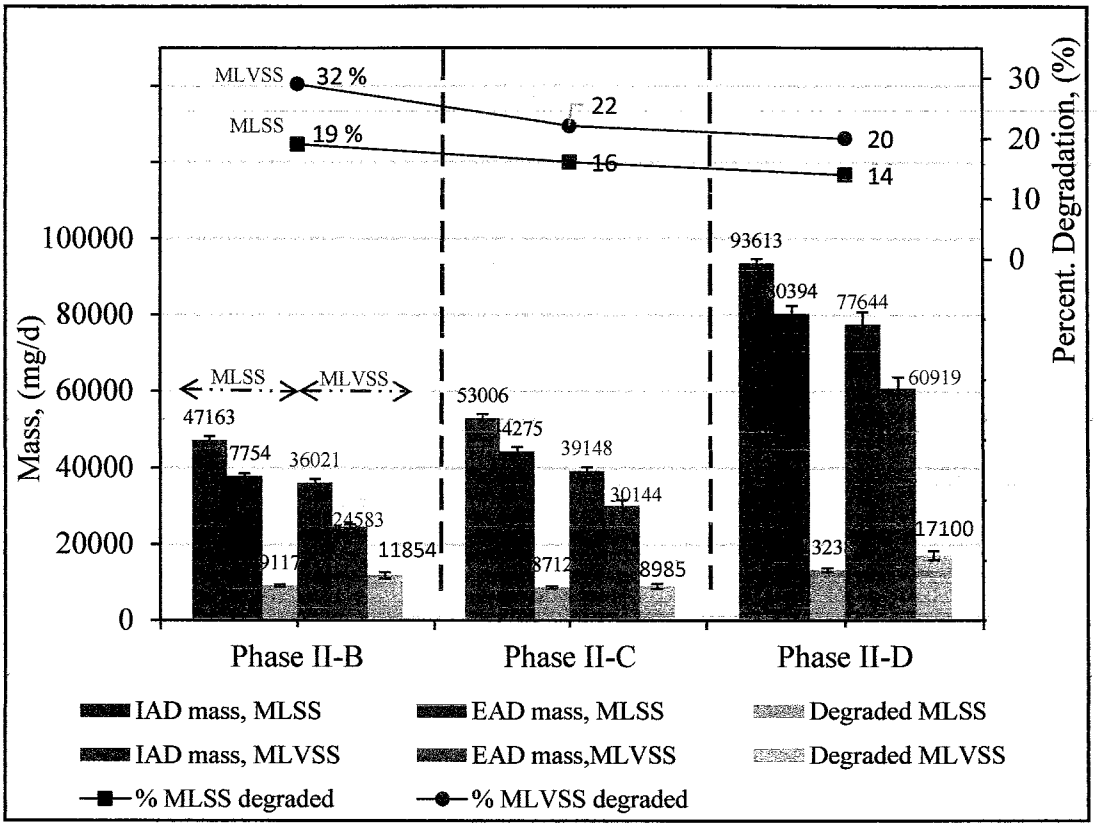


Figure 4.17: Steady state Aerobic Digester Mixed Liquor Suspended and Volatile Solids Mass balance and Degadation Efficiency vs Phases

4.4.3 Phase II results of heterotrophic plate count and population of microfauna

Results for the HPC to determine live heterotrophic bacteria and count for the protozoa and metazoa is presented in this Section. Sludge samples in the aeration chamber were analyzed for HPC and counting of micro-organisms such as protozoa and metazoa.

4.4.3.1 Phase II results for heterotrophic plate count

The main objective of HPC count was to identify the viable bacterial population in the reactor aeration chamber for various MLSS concentrations analysed. The MLSS concentrations tested comprise of 3,000, 3,400, 3,500, 3,700 and 4,800 mg/L. For the determination of the viable bacterial count, 3M Petrifilm Aqua Plate Testing Process method was adopted through direct pipetting of 1 mL serially diluted sludge sample

(10^{-6}) by plating technique, and incubated at 35°C for 48 hours. Samples were filtered with 47 mm; 0.45 micron pore size mixed cellulose Ester (MCE) filter paper. Following the incubation colonies formed were analyzed and counted as indication for the population of microorganisms. The results for counted colonies is presented according to Table 4.4 and Figure 4.18. The microbial growth appearing on specific media was enumerated in terms of HPC cfu/mL (pathogenic indicators).

Table 4.4: Phase II results for heterotrophic plate count of sludge

Aeration tank MLSS, (mg/L)	Heterotrophic plate count, (cfu/mL) x 10^7			
	Sample 1	Sample 2	Sample 3	Average count
3,000	2.4	2.2	1.9	2.17
3,400	3.2	2.6	2.9	2.90
3,500	3.5	3.8	4.1	3.80
3,700	4.4	4.7	5.0	4.70
4,800	5.8	5.3	5.5	5.53

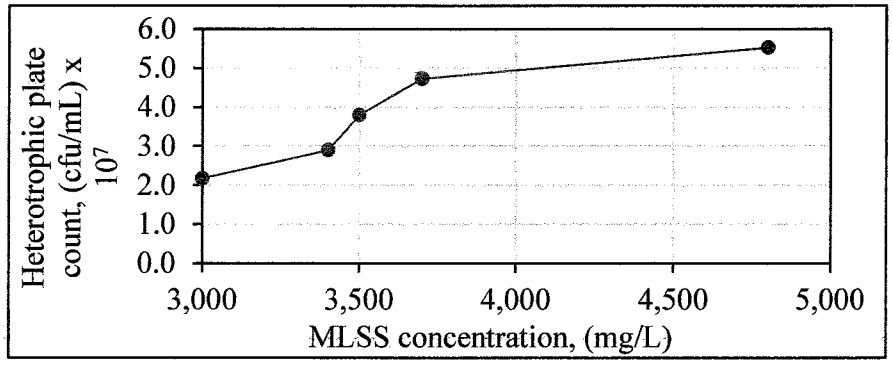


Figure 4.18: Variation of plate counts with aeration chamber sludge

As can be observed from Table 4.4 and Figure 4.18, HPC for respective MLSS concentrations increased with rise in MLSS concentration for the aeration chamber, which probably suggests viability of cells being maintained at higher MLSS concentration in aeration tank. MLSS concentrations of 3,000 mg/L being the minimum tested and MLSS concentration of 4,800 mg/L being the maximum tested yielded HPC average counts of 2.17×10^7 and 5.53×10^7 cfu/mL, respectively. The HPC difference of 3.37×10^7 was observed between lowest MLSS measured (3,000 mg/L) and highest MLSS measued (4,800 mg/L). The equivalent difference in the MLSS concentration was observed to be 1,800 mg/L.

4.4.3.2 Phase II results for protozoa and metazoa count in aeration chamber sludge

The micro-organisms observed at the respective periods is presented in the Table 4.5 as average and percentage. The counting was done in triplicates of 100 µL sub-samples.

Table 4.5: Count for protozoa and metazoa in aeration chamber sludge

Phase II-A	Initial period (Acclimatization)					
Organism	Slide no. 1	Slide no. 2	Slide no. 3	Aver.	Percentage (%)	
Protozoa						
Free-swimming ciliates	8	4	6	6	54.5	81.8
Stalked ciliates	5	3	0	3	27.3	
Metazoa						
Rotifers	1	1	1	1	9.1	18.2
Nematodes	2	1	1	1	9.1	
Total	15	9	8	11	100	100
Phase II-A	Final period (Stable operation)					
Protozoa						
Free-swimming ciliates	6	8	10	8	36.4	91.9
Stalked ciliates	10	9	12	10	54.5	
Metazoa						
Rotifers	2	2	4	3	9.1	9.1
Nematodes	NI	NI	NI	NI	-	
Total	18	19	26	22	100	100
Phase II-D	Final period					
Protozoa						
Free-swimming ciliates	8	10	13	10	33.3	80.0
Stalked ciliates	10	14	17	14	46.7	
Metazoa						
Rotifers	5	3	7	5	16.7	20.0
Nematodes	1	1	NI	1	3.3	
Total	24	28	37	30	100	100
NI = Not identifiable						

Microfauna as protozoa and metazoa were evaluated at the initial and final periods of acclimation in phase II-A, and at end of phase II-D.

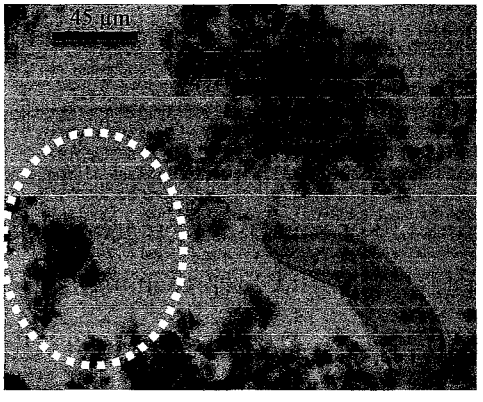
It was observed in the initial period of the acclimatization after the start-up, nematodes were observed to represent 9.0 % (10 organisms/mL) of the microfauna composition. Poor sedimentability of the system was indicated at the begining of the acclimatization which could be due to adaptation of the microorganisms. Rotifers comprised another 9 % (10 organisms/mL) at the begining of the acclimatization period. Total metazoa population was observed to present 18.2 % of the total microfauna. Protozoa such free swimmers (54.5 %) and stalk ciliates (27.3 %) were observed in the reactor after the start up. Population of protozoa was observed to dominate with 81.8 % (90 organisms/mL) of total microfauna. These microorganisms were observed to thrive and were always seen attached to the substrate. The presence of ciliates and rotifer is an indication of good performing reactor.

At the end of phase II-A when the reactor has fully acclimatized, there was an observed increase in the population of rotifers (30 organisms/mL) representing 9.1 % of the total population, free swimming ciliates (80 org/mL) representing 36.4 %, and stalked ciliates (100 org/mL) representing 54.5 %. The protozoa and metazoa population were observed with estimated count of 180 organisms/mL and 30 organisms/mL, respectively. Increased population of rotifers, free swimming ciliates and stalked ciliates could be an indication of possible good enabling environment. Protozoa and metazoa have ability to consume on bacteria and suspended particles as predators, in that way inducing flocculation [312]. Conversely, it was observed that nematodes were the first microorganisms to gradually disappear after the reactor has stabilized in phase II-A. Similar observation was reported by Cordi et al. treating paper mill effluent wastewater using activated sludge process [313].

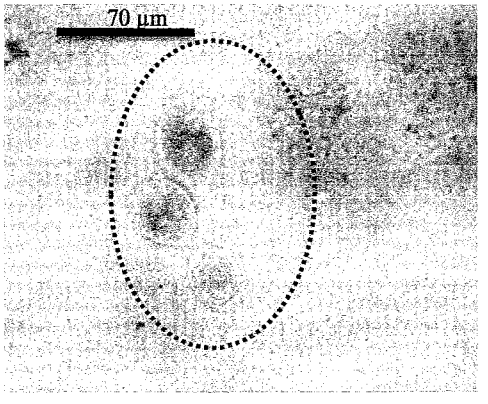
Subsequently, in phase II-D when the SRT was getting older and higher organic loading was operated, increased population of rotifers (50 org/mL) representing 16.7 % was observed. The population of nematodes was observed to reappear with about 10 org/mL having observed percentage of 3.3 % of the total microorganisms. Total observed population of metazoa was 20.0 % (60 org/mL). Similar observations were reported, where population of rotifers and nematodes dominated when operating long SRT for the activated sludge process [314]. Higher percentages of free swimming

ciliates (33.3 %) and stalked ciliates (46.7 %) were also observed as dominant microorganisms representing 80.0 % of the total organisms observed.

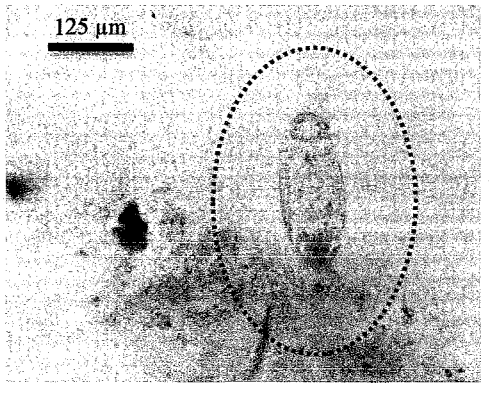
Some noticeable protozoa and metazoan organisms observed to be present at the begining and end of acclimation period in phase II-A and phase II-D are presented in Figure 4.19 . The organisms were viewed under 400x magnification optical microscope. They comprised of free swimming ciliates (Figure 4.19a), stalked ciliates (Figure 4.19b), rotifers (Figure 4.19c) and nemotodes (Figure 4.19d).



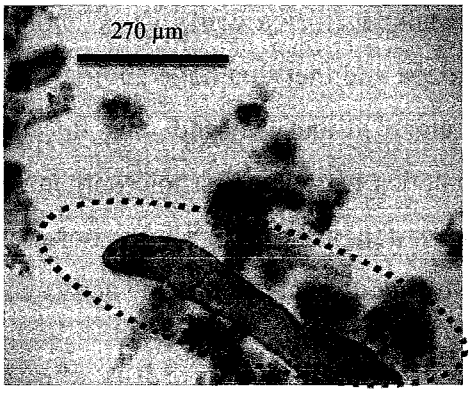
a. Free swimming ciliates (x400)



b. Stalked ciliates (x400)



c. Rotifers (x400)



d. Nemotodes (x400)

Figure 4.19: a, b, c, d illustrate some protozoa and metazoan observed

4.4.4 Phase II results for pH, dissolved oxygen and temperature profiles

pH, DO, and temperature values were monitored and measured in phase II. pH was measured in influent, ANX-C, AER-C and AD-C. DO was monitored and measured

in ANX-C, AER-C, and AD-C. The temperature was measured in situ from AER-C of the i-SGBR system. Complete data for pH, DO and temperature can be found in Appendix B (Table B1.2). To ensure neutral pH was achieved, sodium bicarbonate ($NaHCO_3$) in AER-C and AD-C doses were added. Experiment was conducted at an ambient temperature.

In phase II-A through phase II-D, influent pH for the wastewater was observed to be in the range between 4.2 to 6.8, with average and standard deviation values of 5.89±0.7. The profile for the influent pH in phase II is as shown in Figure 4.20.

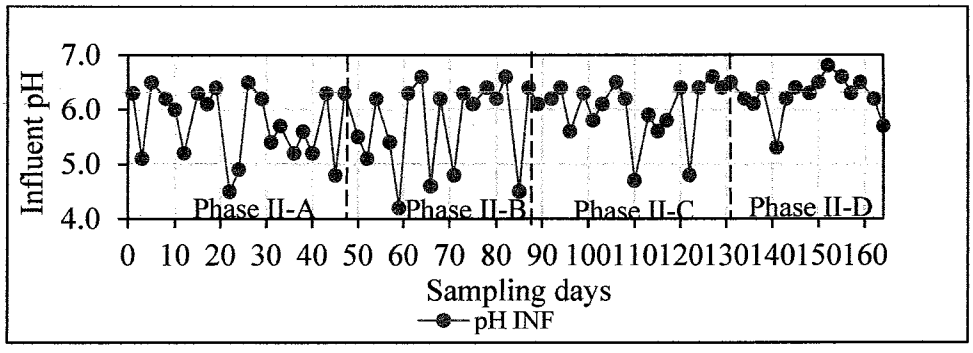


Figure 4.20: pH profile for Influent wastewater vs Sampling Days

pH for ANX-C in phase II-A through phase II-D was observed to range between 7.3-7.8, with average and standard deviation values obtained as 7.45±0.11. pH profile for ANX-C in phase II is shown in Figure 4.21.

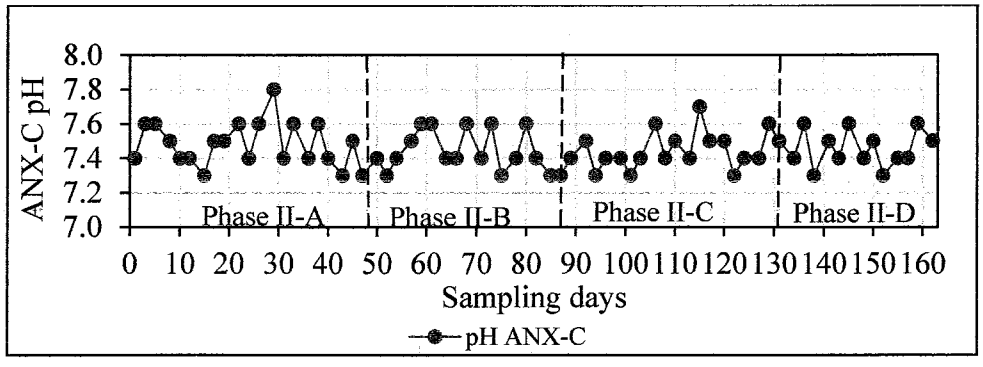


Figure 4.21: pH profile for Anoxic chamber vs Sampling Days

The observed pH for AER-C in phase II-A through phase II-D ranges between 7.2-7.8, with average and standard deviation values obtained as 7.55±0.11. pH profile for the AER-C is as shown in Figure 4.22.

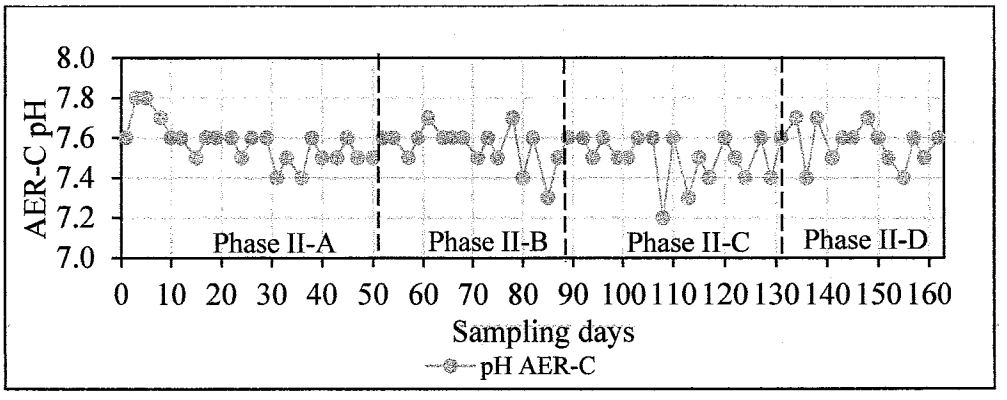


Figure 4.22: pH profile for Anoxic chamber vs Sampling Days

The pH for AD-C in phase II-B through phase II-D was observed to range between 6.8-7.3 with average and standard deviation values obtained as 7.09±0.1. This is shown in Figure 4.23.

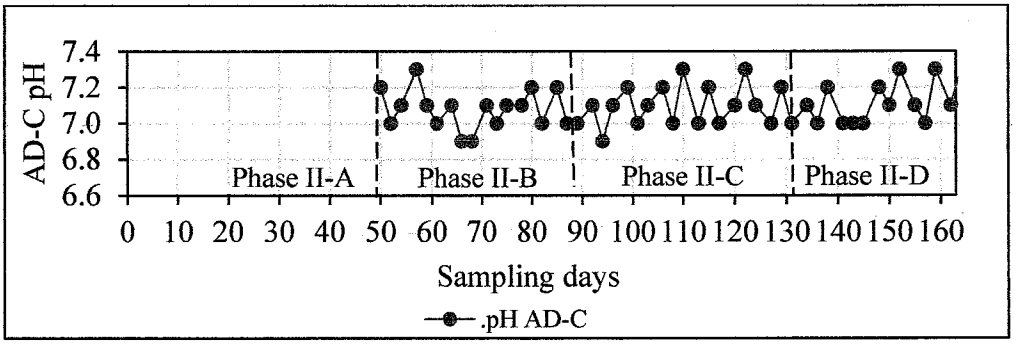


Figure 4.23: pH profile for Aerobic digester vs Sampling Days

It can be observed from Figure 4.21 through Figure 4.23 that neutral pH was maintained in all the reaction chambers during the operation; ANX-C, AER-C, and AD-C throughout the period in phase II. The influent pH of the wastewater was observed to be slightly acidic as can be seen from Figure 4.20. This could be caused due to the nature of the by-products and cleaning operations of the FBI wastewater. Although, the pH in the i-SGBR system was maintained within a stable range of 6.8-7.8, which could be favorable for microbial activities. Tolerable pH for microbial

process range between 6.0 to 9.0 and optimum performance occurs close to neutral pH [89, 90].

DO in the ANX-C for phase II-A through phase II-D ranges between 0.18 mg/L to 0.28 mg/L, with average and standard deviation recorded as 0.23±0.02 mg/L. DO profile for phase II-A through phase II-D in the ANX-C is shown in Figure 4.24.

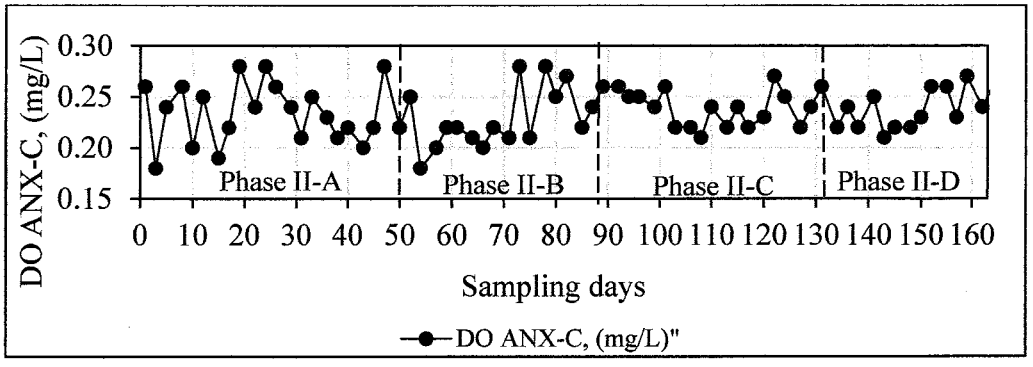


Figure 4.24: Dissolved Oxygen profile for Anoxic vs Sampling Days

DO for AER-C in phase II-A through phase II-D ranges between 3.76 mg/L and 5.75 mg/L with an average and standard deviation of 4.48±0.3 mg/L. DO profile for AER-C for phase II-A through phase II-D is shown in Figure 4.25. Fine bubble diffusers in AER-C serve the dual purpose of providing sufficient mixing and supply of DO.

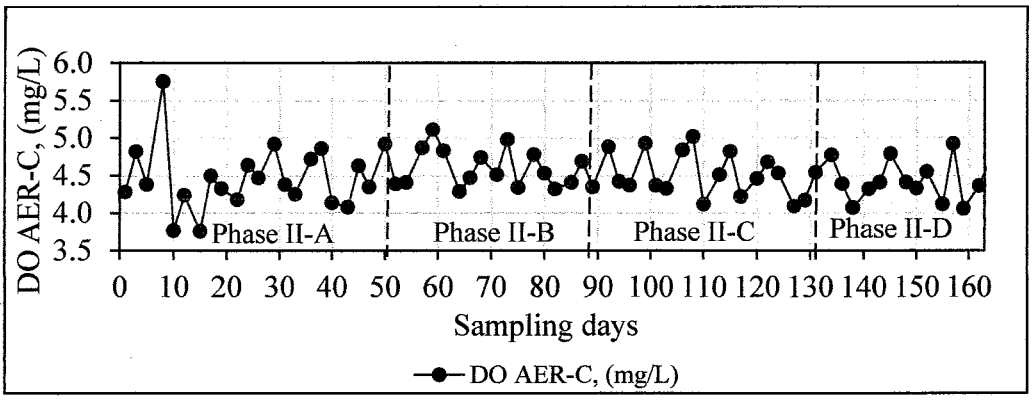


Figure 4.25: Dissolved Oxygen profile for Aeration vs Sampling Days

DO in AD-C between phase II-B through phase II-D was observed in range between 6.93 to 9.33 mg/L with average and standard deviation recorded as 8.28±0.5 mg/L.

Coarse bubble diffusers in AD-C serve the dual purpose of providing sufficient mixing and supply of DO. DO monitoring started from day 50 to day 162 in phase II. DO profiles vs sampling days in AD-C for phase II is shown in Figure 4.26.

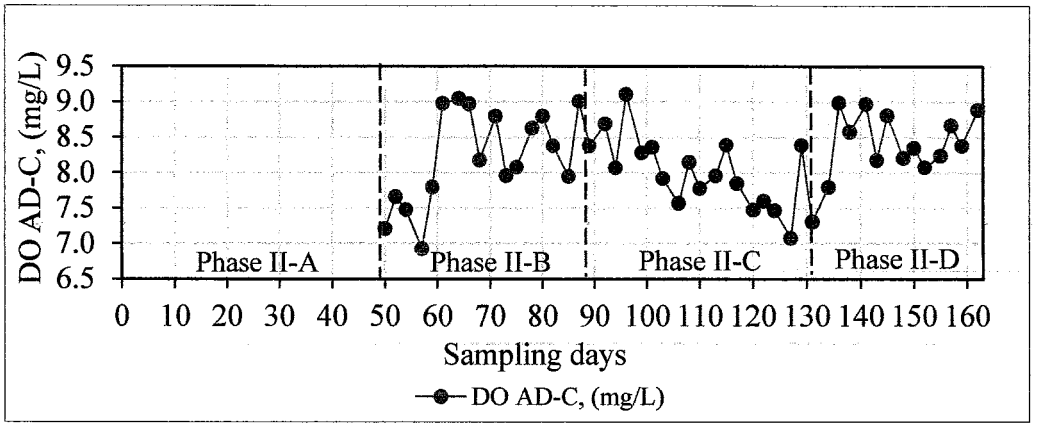


Figure 4.26: Dissolved Oxygen profile for Aerobic Digester vs Sampling Days

The DO fluctuations with standard deviation value of less than 6 % variability might be considered insignificant. However, DO variations might be attributable to requirements for oxidation of the organic matter and nitrification in the aerobic chamber, which resulted in variable influent loadings for the wastewater. DO concentration was always adjusted by gauge valves to ensure required supply.

The temperature was monitored from the AER-C of i-SGBR system in phase II-A through phase II-D, where the observed values range between 27.3 and 33.3 °C. Temperature profile vs sampling days in AER-C is presented in Figure 4.27.

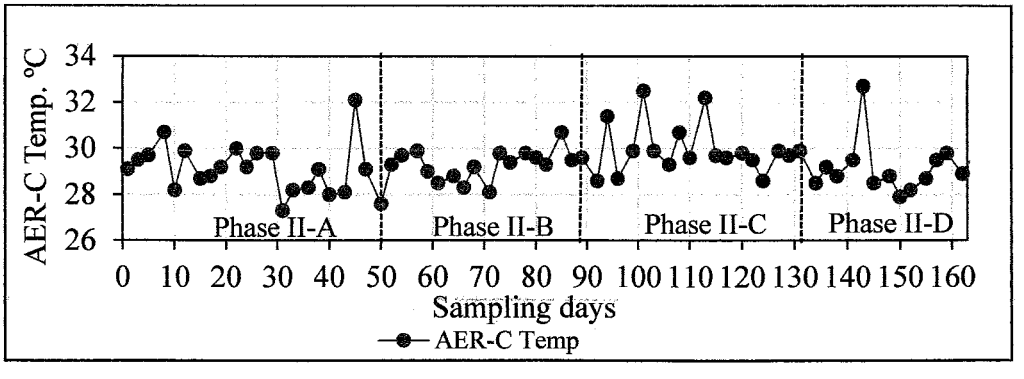


Figure 4.27: Temperature profile for Aeration Chamber vs Sampling Days

The average and standard deviation were recorded as 29.4±1.06 °C. The biochemical reactions could be influenced by temperature.

The growth rate of nitrifying microorganisms is sensitive to temperature [156]. Nitrification can proceed in wastewater at temperatures between 4 to 45 °C, with the nitrification rates reported to rise as a function of temperature [157, 158]. The ammonia and nitrite oxidation was considerably enhanced by increasing temperatures between 10-30 °C [150]. The experimental observations recommended that the effect of temperature on the rate of nitrification can be modeled by an Arrhenius type equation in the range of 7-30 °C (Equation 2.7) [85, 159].

The tropical countries like Malaysia have a mesophilic temperature. Mesophilic temperature is likely to make biological treatment of wastewater favorable [315]. The performance of ASP is very sensitive to temperatures, due to its influence on the rate of biological reactions [240]. The optimum temperatures for bacterial activity are specified to be in the range of 25–35 °C [85, 316]. Phase II results for the system performance: COD, sCOD, and BOD_5.

4.5 Phase II results on performance for the system operation

Results for the biodegradation efficiencies of organic matter (COD, sCOD, BOD_5), nitrogen (TN, ammonia-nitrogen, TKN and nitrate-nitrogen) and TSS were examined by changing the HRT and IR as the main operating variables. The degradation of the organic matter, oxidation of ammonia and reduction of nitrate were achieved through the aerobic process, anoxic process, and aerobic digestion process. To achieve this objective, samples were collected from influent (INF), effluent anoxic chamber (E-ANX), and effluent aeration chamber (E-AER), return activated sludge (RAS), and an effluent clarifier (E-CLAR). The system was operated as an extended aeration activated sludge process. Extended aeration activated sludge performance can best be explained in terms of its ability to remove COD and BOD_5 [317]. Performance data for phase II including paramenters for COD, sCOD, BOD_5, TSS, ammonia-nitrogen, nitrate, nitrite, TKN and TN are contained in Appendix D (Table 1.1 through Table 1.6).

4.5.1 Phase II results for removal of COD, sCOD, and BOD_5

Monitoring and evaluation of organic matter degradation for phase II were carried out in 162 days based on the COD, sCOD, and BOD_5. Experiment in phase II has four sub-phases; phase II-A, phase II-B, phase II-B and phase II-D.

i. Phase II-A was carried out between day 1 to 50, with activities comprising start-up and acclimatization using a flow rate of 45 L/d, IR flow of 135 L/d and 270 L/d. Average values and standard deviation OLR observed for ANX-C and AER-C OLR were 1.34±0.68 kg COD/m^3 d and 0.21±0.03 kg COD/m^3 d, respectively.

ii. Phase II-B was carried out between day 50 to 87 for system operation and simultaneous aerobic digestion, where the flow rate of 45 L/d and IR flow of 270 L/d were operated. Average values and standard deviation observed for ANX-C and AER-C OLR were 1.34±0.68 kg COD/m^3 d and 0.21±0.03 kg COD/m^3 d, respectively.

iii. Phase II-C was carried out between day 87 to 131 with an influent flow rate of 70 L/d and IR flow rate of 420 and 210 L/d. Average values and standard deviation operated for ANX-C and AER-C OLR were observed as 2.26±0.46 kg COD/m^3 d and 0.33±0.03 kg COD/m^3 d, respectively

iv. Phase II-D was carried out from day 131 to 162 with an influent flow rate of 100 L/d and IR flow of 600 L/d. Average values and standard deviation operated for ANX-C and AER-C were observed as 3.05±0.52 kg COD/m^3d and 0.50±0.06 kg COD/m^3 d, respectively

Time-dependent profile for COD: Influent, E-ANX-C, effluent clarifier, and BOD_5: influent and effluent clarifier are shown in Figure 4.28. Phase II operation was carried out for a period of 5.5 months (162 days). For the whole experimental duration, it can be observed that influent wastewater COD concentration fluctuated within the range of 715 mg/L and 1,593 mg/L, with overall average value and standard deviation of 1,223±234 mg/L. It could be seen that the standard deviation value was high, which could be caused due to changes in operational variations and conditions.

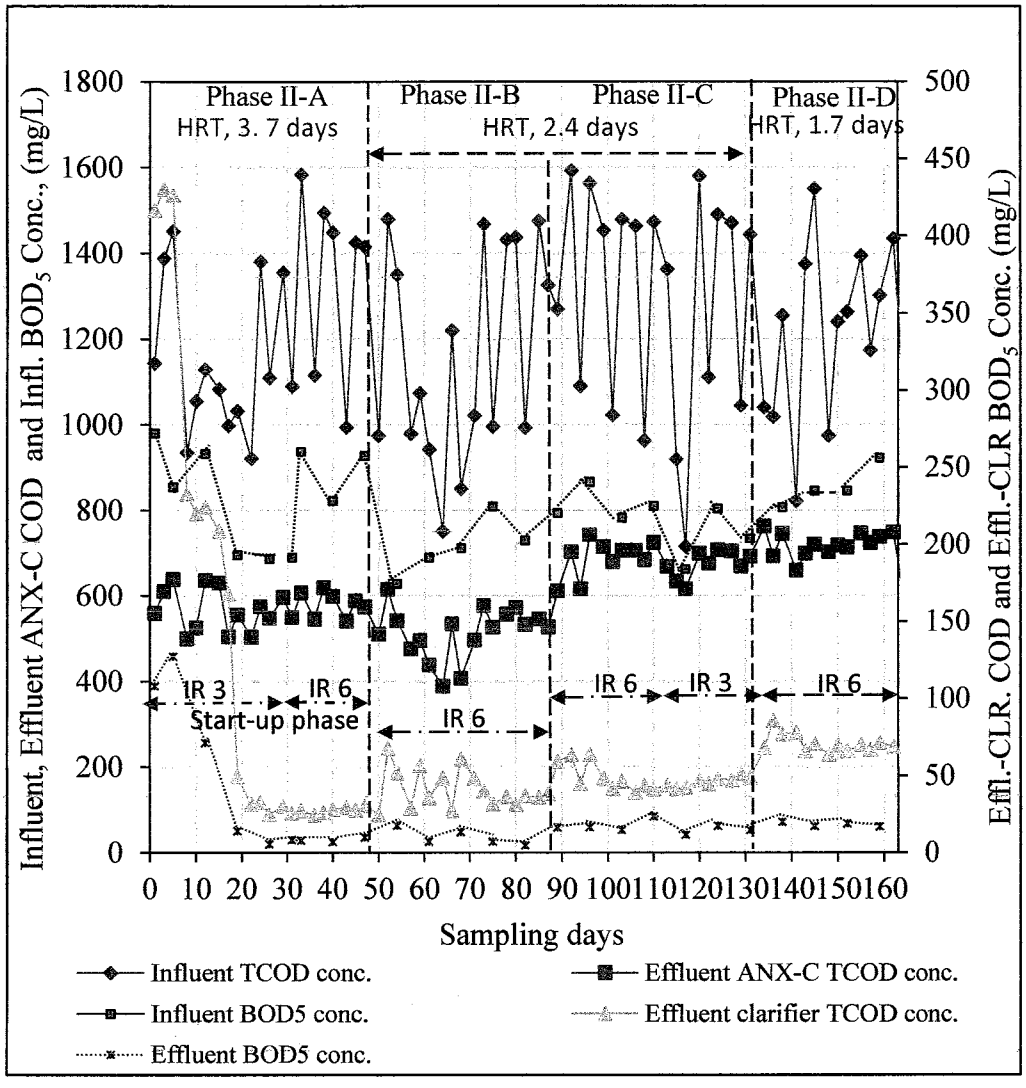


Figure 4.28: Infl., Effl-Anoxic & Effl.-Clarifier COD and Infl. & Effl.-Clarifier BOD_5 Conc. vs Sampling Days

BOD_5 measurement was not the major parameter adopted to analyze degradation of organic matter in this research, yet, BOD_5 was carried out once a week to validate the COD observed values, where measurements for COD parameter was carried out three times per week. Consequently, steady state is not expected for the profile showing BOD_5 time series plot, which was unattained probably due to the time lag compared to COD values. BOD_5 measurements were compared with equivalent COD values to obtain BOD_5/COD ratio on specific days referred and during observed steady state periods.

In phase II-A, it can be seen from Figure 4.28 through Figure 4.29, that COD removal efficiency during the start-up was observed to be low, with less than 80 % percent COD degradation observed between day 1 to day 10, where between day 1 to day 10 average effluent COD concentration was 220 mg/L, which corresponds to average influent COD concentration of 1055 mg/L. High effluent COD could be expected in the beginning due to microbial perturbations through adaptation process to acclimatize. High effluent COD could also be influenced by effluent TSS resulting from suspended biomass. Acclimation period is required for most industrial wastewaters to gradually expose consortium of the microbial community to potential inhibitory compounds. This permits the development of suitable enzyme-producing genes that are necessary to induce biodegradation [84].

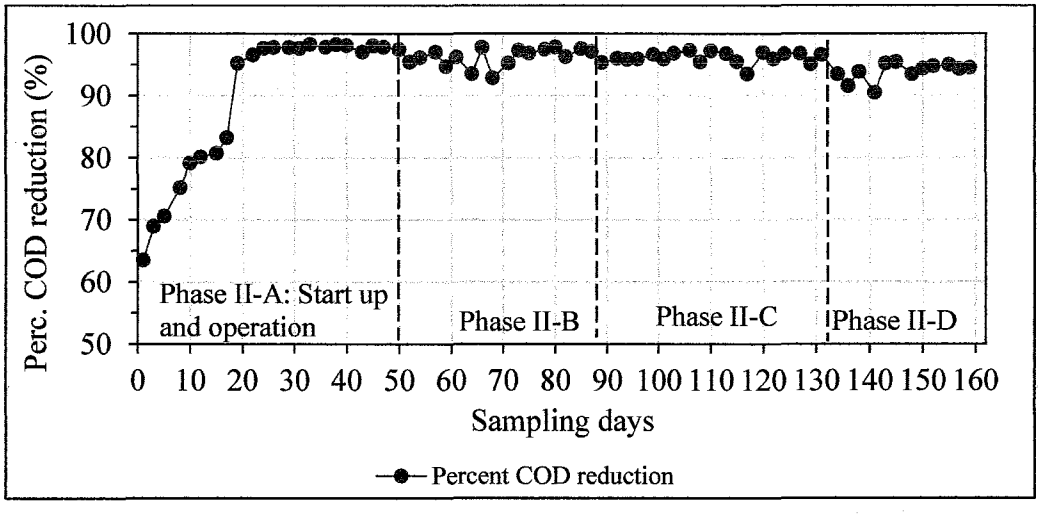


Figure 4.29: Percentage COD reduction vs Samplings Days

However, there was detected and gradual rise in the effluent clarifier COD removal efficiency from 80 % to 95 % between days 12 to day 19. This corresponds to influent and E-CLR average concentrations observed as 1,129 and 224 mg/L, and 1,033 and 49 mg/L, respectively. Equivalent influent and effluent BOD_5 concentration on day 12 and day 19 were 933 mg/L and 71 mg/L, and 695 mg/L and 14 mg/L, respectively. This gives untreated influent concentration and treated effluent BOD_5/ COD ratio on day 12 and 19 as 0.8 and 0.1, and 0.3 and respectively. Between days 22 to day 50, steady state was observed in the E-ANX-C, E-AER-C, and effluent clarifier (E-CLR).

At observed steady state, average values and standard deviation of COD in E-ANX-C and E-CLR were observed to be 571±34 mg/L and 27±3 mg/L, respectively. This gives 97.8 % system removal efficiency with equivalent observed influent COD average and standard deviation values of 1273±219 mg/L. Between day 22 to 50 when steady state was observed, corresponding BOD_5 concentration for influent and E-CLR had observed average and standard deviation values of 804±122 and 8±2 mg/L, respectively. COD removal efficiency of 97.9 % in the system was observed. The untreated influent and treated effluent clarifier BOD_5/COD ratio at steady state period between day 22 to day 50 gives 0.6 and 0.3, respectively. F/M ratio for the AER-C was observed to be 0.03 mg sCOD/mg MLVSS d based on sCOD. It was noticeable that about 55 % of the COD was removed due to biological degradation in ANX-C alone. The specific COD removal rate of 0.304±0.08 mg COD/mg VSS d was observed in the ANX-C. Therefore, since COD is used in the ANX-C for denitrification, the remainder was later degraded aerobically in AER-C. The average specific COD removal rate in the AER-C at the steady state was determined as 0.09±0.25 mg COD/mg VSS d.

In phase, II-B operation was carried out between day 50 to day 87, with the start-up of aerobic digestion on day 50, where E-CLR COD concentration of 22 mg/L was observed before the digestion commenced. On day 54 there was an observed increase in effluent COD concentration of 50 mg/L, which could probably be due to the addition of the solids from effluent AD-C. COD effluent receded in both E-ANX-C and effluent clarifier until day until day 73, when E-ANX-C and effluent clarifier COD were observed to be 577 mg/L and 38 mg/L, respectively. The distortion observed in ANX-C could be due to the operation of the internal recycle. Between day 75 to day 87, steady state was observed for operation with ANX-C and AER-C COD OLR having average value and standard deviation of 1.42±1.66 mg COD/m^3d and 0.20±0.21 mg COD/m^3d, respectively. It was noticed that OLR for ANX-C was higher than the AER-C OLR, which could probably be due to the configuration, having influent COD received first in the ANX-C, and volume ratio of 1:3 for ANX-C:AER-C. Average values and standard deviation for ANX-C and E-CLR COD were observed to be 543±18 mg/L and 35±2.9 mg/L, respectively. The average value and standard deviation for influent COD was observed to be 1276±224 mg/L. Between day 75-87, equivalent average values and standard deviation for the influent and E-CLR

BOD_5 were observed to be 760±69 mg/L and 6±2 mg/L, respectively. This gives untreated and treated BOD_5/COD values of 0.6 and 0.2, respectively. COD removal efficiency of 97.4 % was observed. The F/M ratio in the AER-C was observed to be 0.03 mg sCOD/mg MLVSS d. It can be seen from the observed values of influent COD and E-ANX-C that 57 % of COD has been degraded in the ANX-C. The average value and standard deviation for specific COD removal rate in the ANX-C and AER-C were determined as 0.278±0.1 mg COD/mg VSS d and 0.11±0.3 mg COD/mg VSS d. The F/M was normally low, which could probably be due to large biomass concentration of 2798±152 mg/L operated in the AER-C, the size of the AER-C and utilization of substantial amount of COD in the ANX-C.

In phase II-C, the flow rate of 70 L/d was operated, with equivalent ANX-C and AER-C HRT of 13. 7 hours and 42. 8 hours, respectively. This phase was operated between day 87 to day 131. On day 89, there was an observed increase in E-ANX-C and E-CLR COD concentrations to average values of 612 mg/L and 59 mg/L, respectively. The rise could be due to increased flow rate that resulted in higher ANX-C and AER-C OLR with observed average value and standard deviation of 2.26±0.46 kg COD/m^3.d and 0.33±0.03 kg COD/m^3 d, respectively. IR flow of 420 L/d (IR 6) was operated between day 89 to day 110, and IR flow of 210 L/d (IR 3) was operated between day 113 to day 131. However, there was no observed influence of IR flow on COD removal efficiency observed due to the variation of IR ratio from 100 % to 50 %. Operation with of IR 6 had its steady state from E-ANX-C between day 99 to day 110 with an average value and standard deviation COD of 698±24 mg/L, while operation with IR 3 had it steady state between day 122 to day 131 with an average value and standard deviation COD value of 689±19 mg/L. Although, an observed drop in ANX-C COD concentration was observed on day 113, with the observed value of 662 mg/L. This drop could be due to change in IR flow from 420 L/d to 210 L/d, which probably could induce dilution level in ANX-C in addition to low influent concentration observed between day 113 to day 117, with an observed concentration of 715 mg/L on day 117. This phenomenon has not been noticed in E-AER-C, RAS, and E-CLR. Between day 101 to day 131, steady state was maintained in the effluent clarifier with an average value and standard deviation COD of 45±3 mg/L. Steady state period observed between day 101 to day 131 had observed

influent COD concentration average value and standard deviation of 1,169±349 mg/L, which represents 96 % system COD removal efficiency. The drop in COD percent removal efficiency in phase II-C could be due to drop in ANX-C COD utilization, which was observed at average 42 %, thereby increasing E-CLR concentration. The distortion observed in the operation of ANX-C stability from IR 6 to IR between day113 to day 117 could be due to effect on reduced HRT for ANX-C that may affect microbial activities in the interim. High variability in the influent concentration could be responsible. This phenomenon has not been seen in the effluent clarifier. This could be due to effective heterotropic metabolism in the AER-C and adequate biomass settling in AER-C. The equivalent average values and standard deviation for the influent and clarifier effluent BOD_5 were observed to be 759±62 mg/L and 16±4 mg/L, respectively. This gives untreated and treated BOD_5/COD values of 0.65 and 0.34, respectively.

In phase II-D, the experiment was carried out from day 131 to day 162 with an influent flow rate of 100 L/d, corresponding to ANX-C and AER-C HRT of 9.6 hours and 30 hours, respectively. It can be observed from Figure 4.19 that abrupt fall and the rise of ANX-C and E-CLR concentrations observed on day 136 to day 141 could be due to increase in the ANX-C and AER-C OLR to the average value and standard deviation of 3.05±0.5 kg COD/m^3d and 0.5±0.1 kg COD/m^3d. Hence, between day 143 to day 162, steady state was observed in ANX-C and effluent clarifier, with average values and standard deviation of 733±20 mg/L and 69±2 mg/L, respectively. Equivalent BOD_5 concentration in the effluent clarifier was observed to have average value and standard deviation of 18±1 mg/L. Corresponding influent concentrations for COD and BOD_5 have average values and standard deviation observed to be 1,313±104 and 851±46 mg/L, respectively. This gives untreated and treated BOD_5/COD values of 0.7 and 0.3, respectively. It can be realized that system has achieved 95 % COD removal. The average value and standard deviation for specific COD removal rate in the ANX-C and AER-C were observed to be 0.294±0.12 mg COD/mg VSS d and 0.12±0.05 mg COD/mg VSS d, respectively. Low BOD_5/COD ratio observed could suggest that the wastewater contains high biodegradable organic content.

A statistical analysis (ANOVA) was conducted on the E-CLR COD results obtained from the experimental analysis. At 95% confidence level, the result indicated that there

is a significant difference (P<0.05) between the mean values for the HRT's (Table 4.6), where the results indicated that mean value for HRT with flow rate of 45L/d was lower than the mean values of the other flow rates with 70 and 100 L/d.

Table 4.6: Phase II-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on COD performance

```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.

>> [p,tbl,stats] = anova1(COD)

p =

  1.6165e-010


tbl =

    'Source'     'SS'             'df'    'MS'             'F'           'Prob>F'
    'Columns'    [2.7785e+003]    [ 2]    [1.3893e+003]    [251.0723]    [1.6165e-010]
    'Error'      [    66.4000]    [12]    [     5.5333]          []              []
    'Total'      [2.8449e+003]    [14]                []          []              []


stats =

    gnames: [3x1 char]
         n: [5 5 5]
    source: 'anova1'
     means: [35.6000 48 68.6000]
        df: 12
         s: 2.3523

>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000  -16.3691  -12.4000   -8.4309
    1.0000    3.0000  -36.9691  -33.0000  -29.0309
    2.0000    3.0000  -24.5691  -20.6000  -16.6309


m =

   35.6000    1.0520
   48.0000    1.0520
   68.6000    1.0520
```

It can be concluded that HRT with a flow rate of 45 L/d performed better in terms of COD removal efficiency compared with HRT having flow rates of 70 and 100 L/d. Consequently, the performance decreases with increase in flow rates (decrease in HRT).

The mean values, standard deviation, and system removal efficiencies at steady state are presented in Figure 4.30, where treatment efficiency of 98 % was observed in phase II-A. It can be seen that the treatment efficiency declines with a decrease in HRT although not to a significant level. In phase II-A removal efficiency of 98 % was observed with effluent COD average value of 27 mg/L and average influent COD concentration of 1273 mg/L. In phase II-B, COD removal efficiency of 97 % was observed with E-CLR average COD value of 35 mg/L and average COD influent value of 1,276 mg//L. In phase II-C, average COD removal efficiency of 96 % was observed with influent COD concentration of 1,169 mg/L and E-CLR COD concentration of 45 mg/L. In phase II-D, average COD removal efficiency of 95 % was observed, with influent COD concentration value of 1,313 mg/L and 69 mg/L. The utilization of COD in ANX-C from phase II-A through phase II-D as shown in Figure 4.30. It was observed that COD from influent was utilized at 55 %, 57 %, 42 %, and 79 %, respectively.

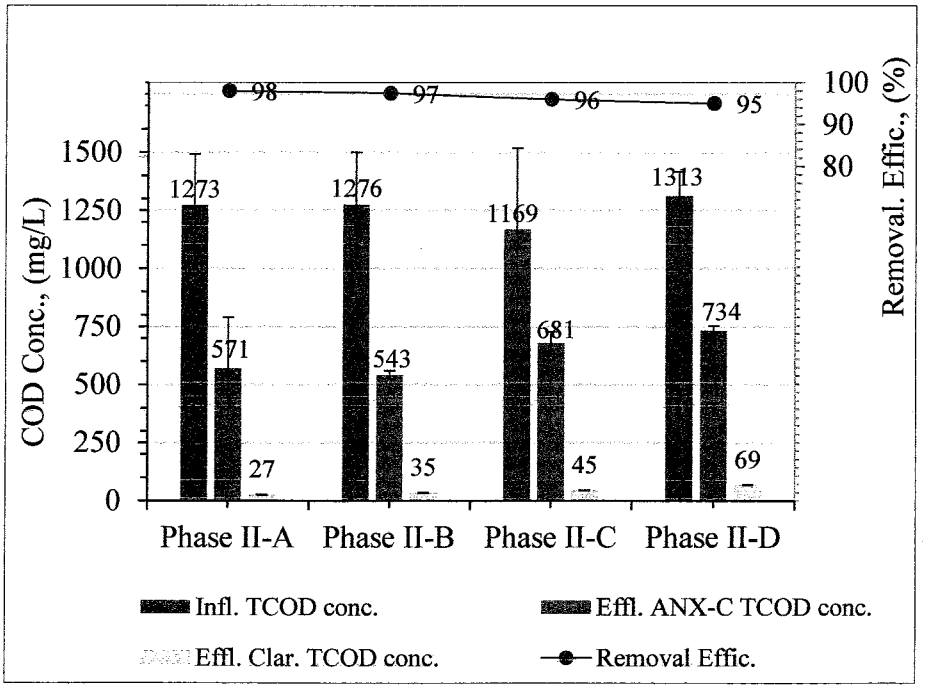


Figure 4.30: Steady state COD results in Infl., Effl.-Anoxic and Effl.-Clarifier vs Phase

4.5.1.1 Phase II results for sCOD concentration reduction profile

The graph for filtered soluble COD (sCOD): influent, E-ANX-C, E-AER-C, RAS, and E-CLR is shown in Figure 4.31. Results obtained for the sCOD samples previously described were used to monitor the sequential decrease of soluble COD removal from the wastewater by heterotropic bacteria. The typical influent sCOD concentration for the duration of 162 days was observed to be in the range of 297 to 591 mg/L, with an average value and standard deviation observed as 475±71 mg/L.

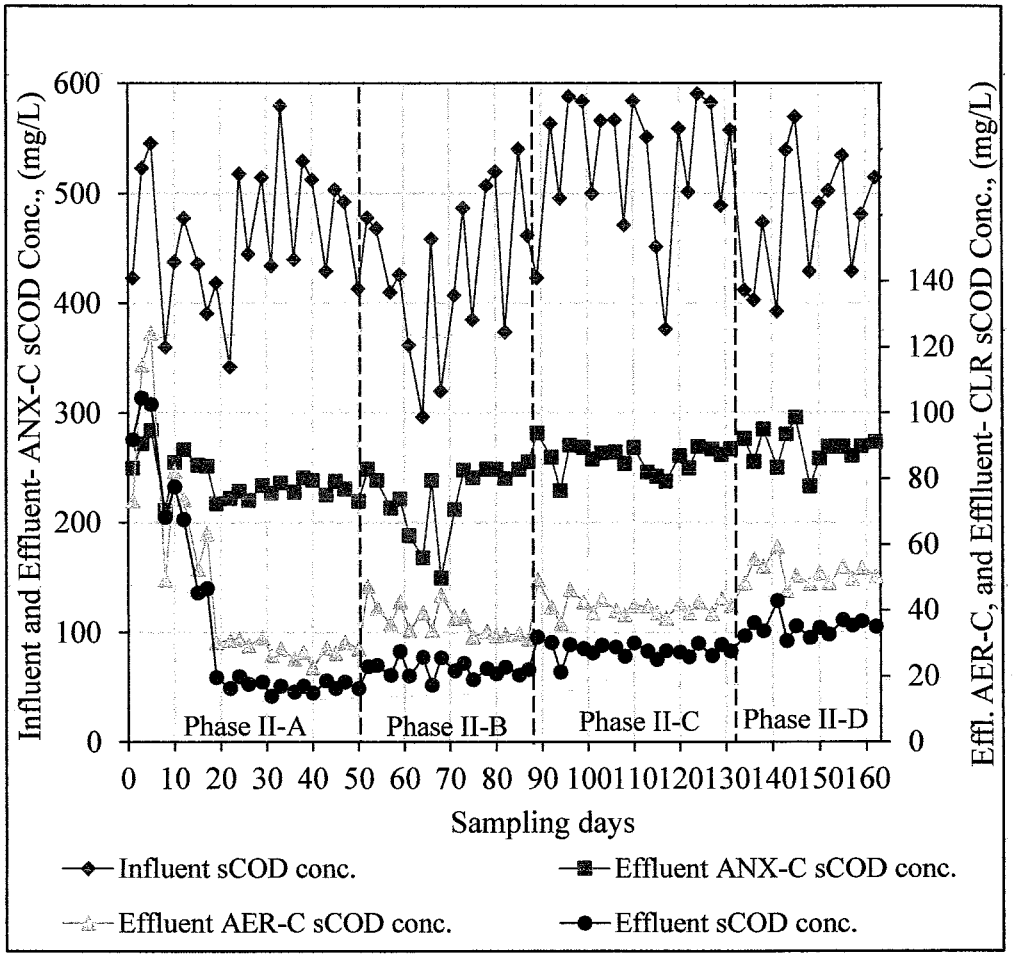


Figure 4.31: sCOD Conc: Infl., Effl.-Anoxic, Effl.-Aeration, & Effl.-Clarifier vs Sampling Days

In phase II-A operation, influent sCOD obtained between day 1 to day 50 was observed to have average value and standard deviation of 462±62 mg/L. This gives influent COD/sCOD ratio of 2.7. Between day 1 to day 17 as observed from Figure 4.31, sCOD concentration in E-ANX-C, AER-C, RAS and effluent clarifier were

not stable, probably due to acclimatization from the start up process. However, between day 22 to day 50, the system has stabilized with observed steady state values measured for E-ANX-C, E-AER-C, RAS and E-CLR, where observed average values and standard deviation were obtained as 231±7 mg/L, 28±2 mg/L, 24±2 mg/L and 17±3 mg/L, respectively. It can be seen that removal efficiency of 96 % sCOD was achieved in the system. The effluent COD/sCOD ratio was observed as 1.6. It can be noticed that the observed BOD_5 values were lower than the sCOD values, which could indicate complete oxidation of the organic matter. Reduction of sCOD was observed in RAS to a value of about 4 mg sCOD/L from E-AER-C, although insignificant, but could indicate possible metabolic process taking place in the clarifier, likely due to a large amount of biomass that has thickened.

Operation in phase II-B was carried out between day 52 to day 87 with a flow rate of 45 L/d as the previous phase. On day 50, aerobic digestion started, and on day 54 there was an observed increase in the sCOD in E-ANX-C, AER-C, RAS and effluent clarifier to have average values of 249 mg/L, 47 mg/L, 36 mg/L and 27 mg/L, respectively. The increase in the sCOD could be due to sludge degradation with its effluent emptied into the AER-C, and IR of nitrates mixed liquor into the ANX-C. Between day 75 to day 87, steady state was observed. The average influent value of sCOD concentration operated at steady was observed to be 459±67 mg/L. This gives COD/sCOD ratio of 2.8. Average values and standard deviation in E-ANX-C, E-AER-C, RAS and effluent clarifier were observed as 250±11 mg/L, 35±6 mg/L, 29±4 mg/L and 22±4 mg/L, respectively. The removal efficiency of 94 % sCOD was observed for the whole system. The effluent COD/sCOD ratio was observed to be 1.6. Similar observation was made for the reduction of sCOD in RAS with the difference having an average concentration of 7 mg/L compared to effluent clarifier concentration. However, no noticeable effect was seen for any poor sludge settlement performance in the clarifier, which might have abated due to daily sludge feed into the AD-C and RAS flow to AER-C in maintaining biomass concentration.

In phase II-C, the flow rate was increased to 70 L/d from 45 L/d, with observed upsurge seen in the E-ANX-C sCOD concentration on day 89 with an average value of 274 mg/L. Similar increase on day 89 was observed for rise of sCOD in E-CLR-C,

RAS and E-CLR with average values observed as 49, 39 and 32 mg/L respectively. This rise could be due to disruption of the microbial activities due to increased flow rate and reduction of ANX-C and AER-C HRT to 13.7 hours and 42.8 hours, respectively. Between day 89 to day 131, two regimes of IR flow were operated. IR flow of 420 L/d (IR 6) was operated between day 89 to day 110, and IR flow of 210 L/d (IR 3) was operated between day 113 to day 131. Steady state was observed between day 101 to day 131 in E-AER-C, RAS and E-CLR, with average values and standard deviation observed as 257±11 mg/L, 41±2 mg/L, 35±2 mg/L and 27±2 mg/L, respectively. This gives effluent COD/sCOD ratio of 1.7. There was no observed effect noticed between day 87 to day 131 to influence the removal of COD, due to IR flow operated with 420 L/d and 210 L/d. Equivalent sCOD influent concentration observed for the operation in steady state between day 101 to day 131 was 520±68 mg/L. This indicates removal efficiency of system sCOD concentration of 94 %.

In phase II-D, the flow rate was increased to 100 L/d, with the corresponding decrease in the ANX-C and AER-C HRT to 9.6 hours and 30 hours. A slight rise of E-ANX-C concentration was observed on day 134 with an average concentration of 277 mg/L. The corresponding influent sCOD concentration observed on day 134 was 412 mg/L. On day 134, it was similarly observed that sCOD concentration for E-AER-C, RAS, and E-CLR were elevated to average values of 48, 42 and 32 mg/L, respectively. This increase of sCOD concentration observed could be associated to increase of flow rate, thereby likely reducing the contact time between the wastewater and microorganism, with a subsequent decrease in both ANX-C and AER-C HRT. Between days 143 to day 162, steady state was observed in E-ANX-C, E-AER-C and E-CLR with average values and standard deviation of 268±17 mg/L, 50±3 mg/L, 44 ±4 mg/L and 34 ±3 mg/L, respectively. Influent sCOD concentration operated for the steady state was observed to have average value and standard deviation of 499±78 mg/L. The system sCOD removal efficiency was observed to be 92 %. The ratio of influent and effluent COD/sCOD gives 2.6 and 2.0, respectively. In this phase, the operation was carried out to build up biomass from average of 3727 mg/L on day 134 to average of 5,273 mg/L on day 159. Influent COD/sCOD gives a ratio of 2.2.

A statistical analysis (ANOVA) was conducted on the effluent clarifier sCOD results taken from the experimental data. At 95% confidence level, the result has shown that significant difference ($P<0.05$) exist between the mean values of the HRT's (Table 4.7).

Table 4.7: Phase II-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on sCOD performance

```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.

>> [p,tbl,stats] = anova1 (SCODEAERPH11)

p =

  1.3096e-006


tbl =

    'Source'      'SS'          'df'    'MS'          'F'          'Prob>F'
    'Columns'     [512.8373]    [ 2]    [256.4186]    [31.5411]    [1.3096e-006]
    'Error'       [146.3342]    [18]    [  8.1297]          []             []
    'Total'       [659.1715]    [20]            []          []             []


stats =

    gnames: [3x1 char]
         n: [7 7 7]
    source: 'anova1'
     means: [22.7286 27.7714 34.7800]
        df: 18
         s: 2.8513

>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -8.9325   -5.0429   -1.1532
    1.0000    3.0000  -15.9411  -12.0514   -8.1618
    2.0000    3.0000  -10.8982   -7.0086   -3.1189


m =

   22.7286    1.0777
   27.7714    1.0777
   34.7800    1.0777
```

The results indicated that the mean value for HRT with flow rate of 45 L/d was lower than the mean values of other flow rates having 70 L/d and 100 L/d. It can be concluded that HRT with a flow rate of 45 L/d achieved better performance of sCOD removal efficiency compared to HRT with flow rates of 70 L/d and 100 L/d. Subsequently, the performance decreases with increase in flow rates and decrease in HRT.

The steady state results for phase II sCOD are shown in Figure 4.32, where presentation of the average observed values are plotted for sCOD with steady state data in influent, E-ANX-C, E-AER-C, RAS and E-CLR for phase II-A through phase II-D.

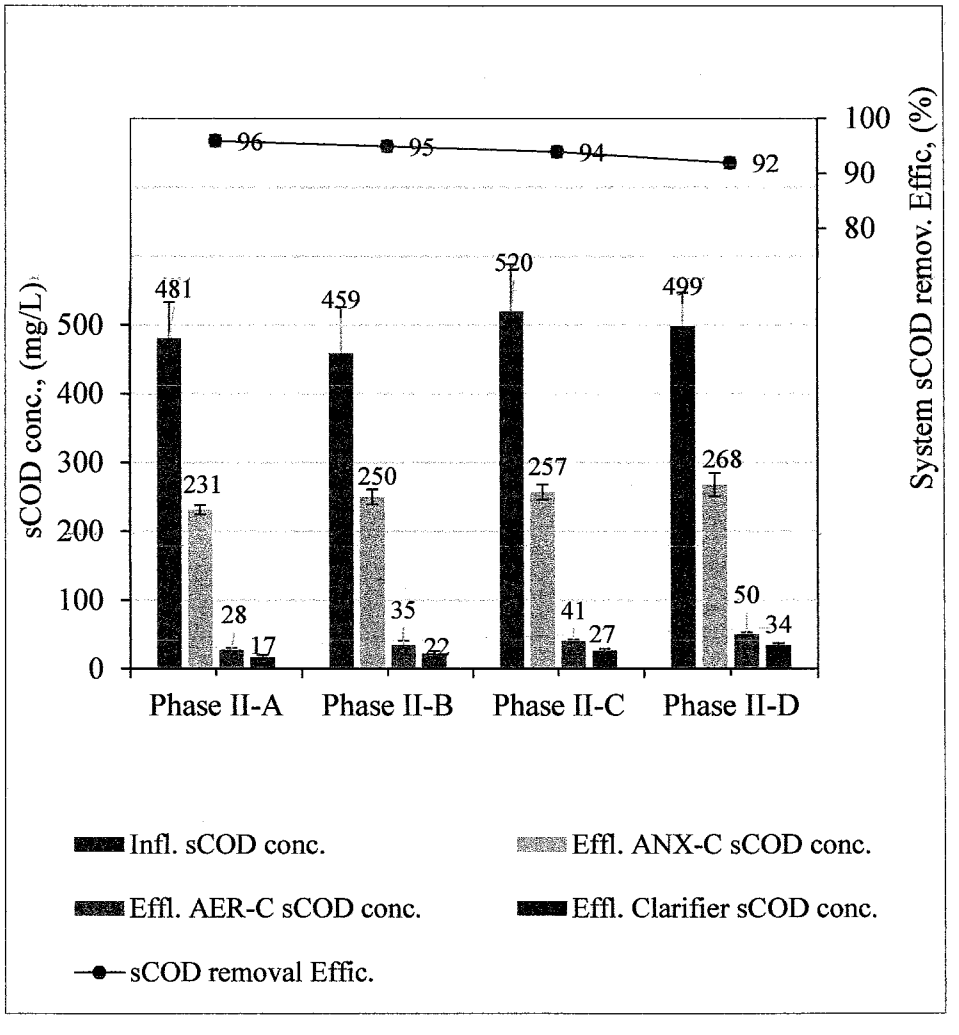


Figure 4.32: Steady state sCOD Conc: Infl., Effl.-Anoxic, Effl.-Aeration, and Effl.-Clarifier vs Phases

4.5.2 Phase II results for the removal of Total suspended solids

The influent and effluent total suspended solids data over the time course of study in phase II is shown in Figure 4.33. TSS value in phase II typically range between 360 to 788 mg/L, with average value and standard deviation of 614±107 mg/L.

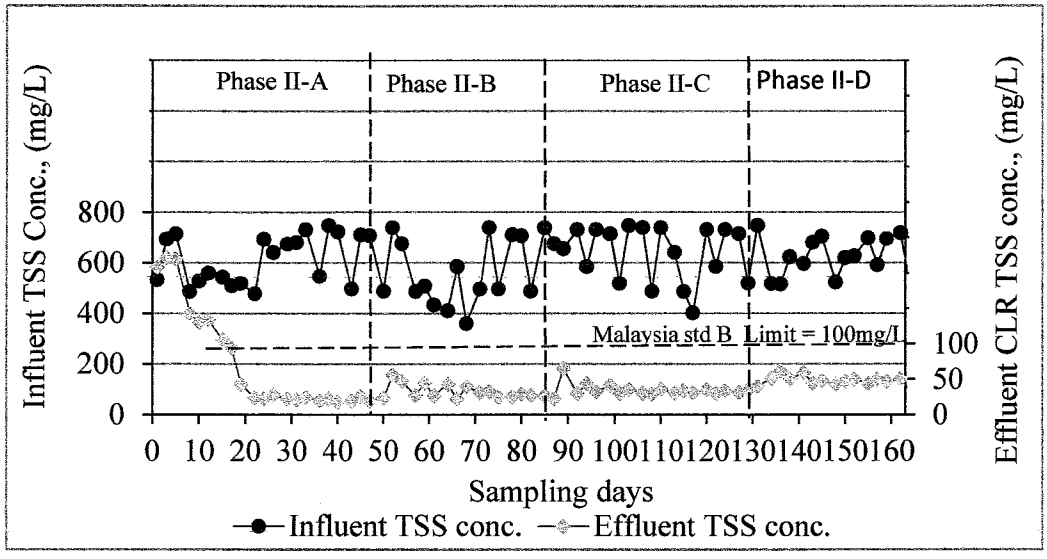


Figure 4.33: Influent and Effluent Clarifier TSS Conc. vs Sampling Days

The steady state results for phase II influent and effluent TSS profile according to Figure 4.33 is interpreted accordingly in Figure 4.34. The error bar represents standard deviation.

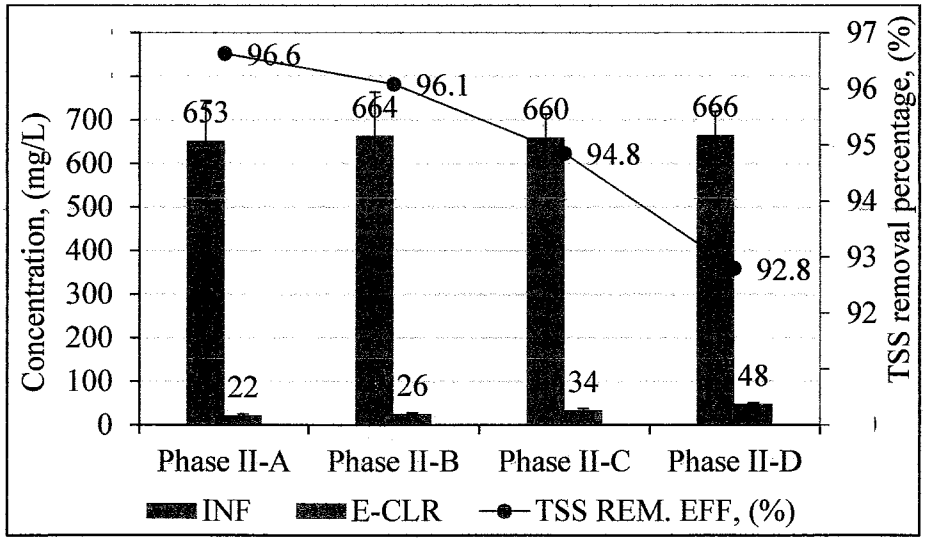


Figure 4.34: Steady state results for Infl. and Effl.-Clarifier TSS Conc. vs Phases

Phase II-A was operated between day 1 to day 50 to start up and stabilize the reactor system. In this study, acclimation was considered complete with effluent COD and effluent TSS having relative constant values after 22 days of operation. It can be observed that there was initially high effluent TSS of up to 99 mg/L on day 15 after the start-up of the reactor due to the probable loss of biomass in the effluent. This could be due to biomass still needed time to adapt in the reactor to achieve adequate settling. This situation possibly resulted in high effluent COD observed during the similar period with an equivalent COD value of 209 mg/L on day 15. Between days 22 to day 50, steady state was observed, with effluent TSS average value and standard deviation observed as 16±2.9 mg/L. Influent TSS for the equivalent period of steady state between days 22 to day 50 was observed to be 640±100 mg/L. This gives COD/TSS ratio for the influent and effluent as 2.1 and 1.7, respectively.

Phase II-B was operated between day 50 to day 87 to include aerobic digestion of sludge. Monitoring of AD-C effluent commenced on day 52, with an observed rise in effluent TSS concentration seen on day 52 with an average value of 49 mg/L. This rise could be due to effluent COD from the AD-C added to initiate elevated solids concentration in the system due to digested sludge. Between day 75 to day 87, steady state was observed, where effluent TSS obtained with an average value and standard deviation of 26±2.3 mg/L. Corresponding influent TSS concentration was observed to be 664±101 mg/L. This gives influent and effluent COD/TSS ratio of 1.3 and 2.2, respectively. Larger ratio of COD/TSS in effluent could suggest low effluent TSS, likely due to improvement in solids settling in the AD-C, or high content of biodegradable organic matter that was consumed by microorganisms in the reactor system.

In phase II-C operation, the surge in TSS concentration level was noticed on day 89, which could be due to increase in the OLR subsequent to increased flow rate from 45 L/d to 70 L/d. Effluent TSS from day 87 to day 99 was noticed to be irregular. However, between day 101 to day 131, steady state was observed, with observed average value and standard deviation of 34±3.9 mg/L. Equivalent influent average value and the standard deviation were observed to be 660 ±101 mg/L. The variability

is high, although, it could be due to the nature of the variable influent wastewater concentration. This gives influent and effluent COD/TSS ratio of 1.8 and 1.3.

Phase II-D was operated between day 131 to day 162 with flow rate of 100 L/d. This indicates an increased organic loading rate with anoxic and aerobic HRT of 3.05±0.52 kg COD/m^3d and 0.50±0.06 kg/m^3d, respectively. Between day 134 and day 141, there were instabilities in the effluent concentrations, although with a peak concentration of 62 mg/L observed on day 136. These instabilities could be due to increased loading and reduced HRT. Between day 143 to day 162, steady state was observed for effluentt TSS, with an average value and standard deviation observed as 48±3.0 mg/L. The effluent concentration of 48±3.0 mg/L represents removal efficiency of 94.6 %. The observed effluent TSS efficiency was comparatively lower than performance with flow rates of 45 L/d and 70 L/d, where removal efficiencies of 96.4 % and 94.6 were observed.

Statistical analysis (ANOVA) was conducted on the effluent clarifier TSS results obtained from the experimental data (Table 4.8). At 95% confidence level, the result has shown that significant difference ($P<0.05$) between the mean values of the TSS exist for the respective HRT's operated, where the results indicated that the mean value for HRT with flow rate of 45 L/d was lower than the mean values of other flow rates having 70 L/d and 100 L/d.

It can be concluded that HRT with a flow rate of 45 L/d achieved better performance of TSS removal compared to HRT with flow rates of 70 L/d and 100 L/d. Consequently, gradual decline in performance of TSS removal efficiency was observed with gradual increase in OLR and decrease in HRT.

Table 4.8: Phase II-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on TSS performance

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Command Window
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>> [p,tbl,stats] = anova1(TSS1)

p =

  4.1091e-007


tbl =

    'Source'     'SS'             'df'    'MS'          'F'          'Prob>F'
    'Columns'    [1.2929e+003]    [ 2]    [646.4667]    [63.5869]    [4.1091e-007]
    'Error'      [       122]     [12]    [ 10.1667]          []             []
    'Total'      [1.4149e+003]    [14]            []          []             []


stats =

    gnames: [3x1 char]
         n: [5 5 5]
    source: 'anova1'
     means: [26 33.8000 48.4000]
        df: 12
         s: 3.1885

>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000  -13.1800   -7.8000   -2.4200
    1.0000    3.0000  -27.7800  -22.4000  -17.0200
    2.0000    3.0000  -19.9800  -14.6000   -9.2200


m =

   26.0000    1.4259
   33.8000    1.4259
   48.4000    1.4259
```

4.5.3 Phase II results for the removal of nitrogen

In wastewater treatment process, nitrogen could be removed either through assimilation into the biomass or by biological nitrification under aerobic conditions and denitrification process under anoxic conditions by maintaining depleted levels of oxygen concentration [84]. In modern decades, biological nitrification-denitrification process has been commonly used for ammonia-nitrogen and TN removal [318]. This process involves two stages:

(a). Conversion of ammonium into nitrate as nitrification process; and

(b). Subsequent transformation of nitrate to nitrogen gas as denitrification [122].

Removal of ammonia-nitrogen, nitrate-nitrogen, TKN and TN are presented in this Section. Ammonia-nitrogen, TKN, and TN were monitored in influent, E-AER-C and E-CLR. Nitrate-nitrogen was monitored in influent, E-ANX-C, E-AER-C, and E-CLR.

4.5.3.1 Phase II results for removal of Ammonia-nitrogen

Nitrification performance of the system was assessed based the removal of ammonia-nitrogen. Dissolved oxygen above 2 mg/L is required for nitrification process. Nitrification process is an autotrophic process where nitrifying bacteria utilize oxygen at a much slower rate than heterotrophic bacteria utilizes oxygen in the removal of carbonaceous organic matter [142]. Nitrifying bacteria are primarily obligate autotrophs, which consume carbon dioxide as their primary carbon source, and obligate aerobes, which require oxygen to grow [319]. Profile for the trend and transformation of the ammonia nitrogen concentration in the reactor system is presented in Figure 4.35.

The graph shows the ammonia-nitrogen concentration in influent, RAS and E-CLR, and ammonia loading rate (ALR) for phase II. Phase II study was conducted in 162 days. Throughout the duration of the phase II study, minimum, maximum, average value and standard deviation for an influent feed of the ammonia nitrogen concentration was observed as 39.1 mg/L, 59.3 mg/L and 51±5 mg/L, respectively.

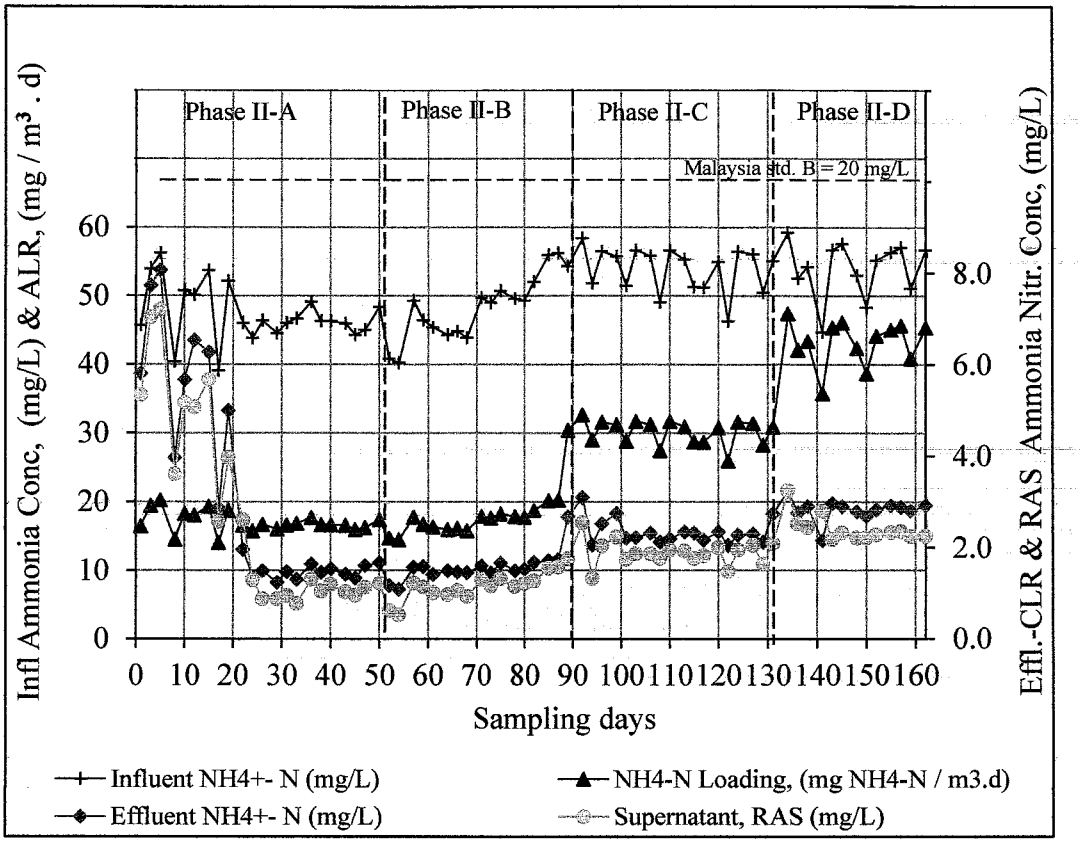


Figure 4.35: Ammonia-Nitr. Conc. Infl., Return Activated Sludge, Effl.-Clarifier and Ammonia Loading Rate vs Sampling Days

Evaluation of system for performance on nitrification efficiencies (η_N) and nitrification rates (r_N) was carried out in phase II. Estimation of ηN was done based on the difference of influent and effluent ammonia nitrogen concentration according to Equation 3.26. Calculations for the observed r_N were done based on total mass of ammonia nitrogen (mg) removed with respect to MLVSS (g) existing in the AER-C expressed as mg NH_4-N/g VSS d according to Equation 3.29. Although, the formation of ammonia-nitrogen is expected from the oxidation of organic matter in the AER-C, however, it is expected the ammonia-nitrogen is reduced to nitrate, which is subsequently reduced to nitrogen gas in ANX-C. The results interpretation for Figure 4.35 is detailed according to Figure 4.36, where influent, effluent ammonia nitrogen and nitrification efficiency are plotted from phase II-A through phase II-D. The error bar represents standard deviation.

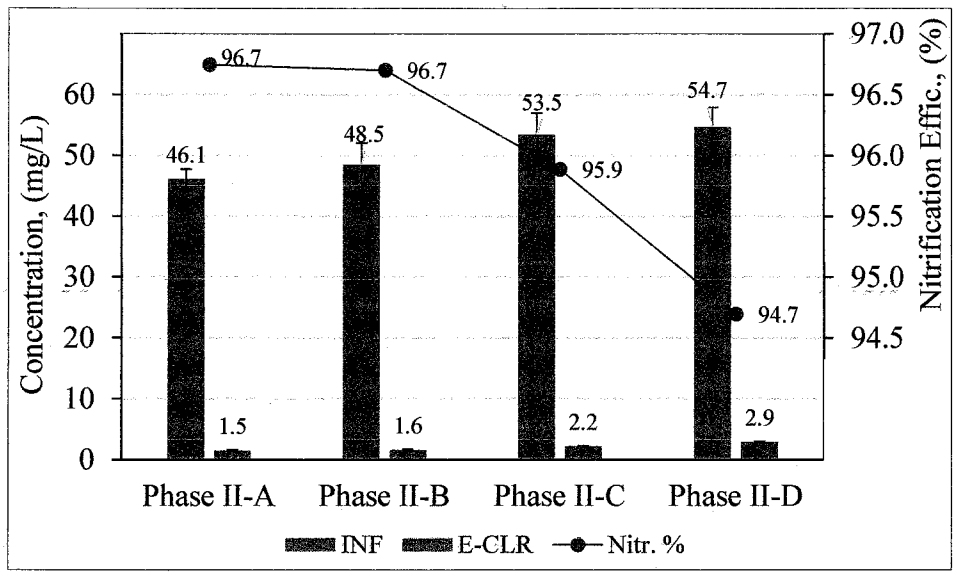


Figure 4.36: Steady state results for Ammonia Nitrogen Conc. in Infl., Effl.-Clarifier and Nitrification efficiency vs. Phases

In phase II-A, there were fluctuations observed during the start-up process from days 1 to 22, which could be due to acclimatization and adaptation process of the micro organisms. Nitrifiers are slow growers, hence need an enabling environment for effective growth. Between days 24 to day 50, steady state was observed. Observed average value and standard deviation for the ammonia loading rate (ALR) during the steady state period observed between days 24 to day 50 was 16.6±0.6 mg NH_3-N/m^3 d. ALR represents the ammonia nitrogen load in the influent in relation to the volume of AER-C. The operation with this ALR yielded average value and standard deviation for the E-CLR as 1.5±0.1 mg/L with observed η_N of 96.8±0.2 %. The corresponding influent ammonia nitrogen concentration was observed to be 46.1±1.6 mg/L as an average value and standard deviation. The experimental influent COD/N ratio for the steady state period based on influent TN concentration was observed to be 17.4, with observed r_N average value and standard deviation of 6.2±0.5 mg NH_3-N/g VSS d. According to Carrera et al. [122], COD/N affects r_N as a result of competition for substrate between heterotrophic and autotrophic bacteria. An observed increase was noticed in the E-CLR from the measured ammonia nitrogen supernatant in RAS obtained as 1.1±0.2 mg/L. This could likely be due to the conversion of organic nitrogen to ammonia nitrogen in the CLR considering a large amount of biomass present.

In phase II-B, a drop in concentration was observed in the E-CLR on day 52 when the monitoring of effluent aerobic AD-C started. The influent and E-CLR average values were observed to be 40.8 mg/L and 1.17 mg/L, respectively. This occurrence could be caused by the dilution effect from the EAD sludge into the AER-C, which was expected to contain little or no ammonia nitrogen in anticipation of full oxidation of ammonia-nitrogen to nitrate. It can be observed that the operation of AD-C has affected the stability of COD and TSS between days 50 to day 73 due to probable solids from the operation of AD-C before stabilization. However, this has not been observed for the trend with ammonia nitrogen. Between days 57 to day 87, steady state was observed with E-CLR average value and standard deviation of 1.6±0.1 mg/L. Similar phenomenon for an increase in ammonia nitrogen concentration was observed, where RAS ammonia nitrogen of 1.2±0.2 mg/L was observed. The average value and standard deviation for ALR after the steady state with aerobic digestion was observed as 17.4±1.3 mg NH_3-N/m^3 d. The corresponding influent ammonia nitrogen concentration was observed as 48.5±3.6 mg/L. The observed η_N was 96.8±0.1 %, and r_N was observed to have average value and standard deviation of 6.2±0.5 mg NH_3-N/g VSS d. Corresponding influent COD/N ratio was observed to be 12.5. IR ratio of 3 for phase II-A and 6 for phase II-B were not seen to influence performance of ammonia nitrogen removal efficiency.

In phase II-C, the operation was performed between days 87 to 131 with increased flow rate from 45 L/d in phase II-B to 70 L/d in phase II-C. There was an observed fluctuation between days 89 and day 103, which could be due to increased flow and reduced HRT. AER-C was observed to be 42.8 hours and system HRT of 1.43 days. HRT in phase II-B for AER-C was observed to be 66.7 hours, with system HRT of 5.89 hours. Between day 73 to day 131, steady state was observed, with operated ALR having average value and standard deviation of 29.9±2 mg NH_3-N/m^3 d and influent concentration average value and standard deviation of 53.5±3.5 mg/L. Corresponding E-CLR average value and concentration of 2.2±0.1 mg/L was observed, and 95.8±0.2 % η_N was achieved. The concentration of RAS supernatant ammonia concentration was observed to be 1.8±0.2 mg/L, which can be seen to be lower than the E-CLR concentration, signifying probable conversion of organic ammonia to ammonia nitrogen. Influent COD/N ratio was observed to be 21.4 and r_N was observed to have

An average value and standard deviation of 9.8±0.7 mg NH_3-N/g VSS d. It was observed that operation of IR ratio of 6 and 3 between day 89 to day 110, and days 113 to 131 was not seen to impact on ammonia nitrogen removal efficiency.

In phase II-D, the flow rate of 100 L/d was operated and HRT reduced from phase II-C operation to 1.25 days for the system and 30 hours for the AER-C. Between day 89 to day 141, instabilities in steadiness were observed possibly due to increase in ALR. Between days 143 to 162, steady state was observed. At steady state, the average value and standard deviation for η_N was observed to be 94.7±0.18 %, with corresponding influent and E-CLR average concentrations values and standard deviation of 54.7±3.2 and 2.86±0.1 mg/L, respectively. Slight increase in ammonia nitrogen concentration was observed from RAS, where RAS observed concentration had average value and standard deviation of 2.29±0.1 mg/L. Corresponding ALR was observed to be 43.8±2.3 mg NH_3-N/m^3 d. The influent COD/N ratio was observed to be 5.6, where r_N was observed to have average value and standard deviation of 11.4±2 mg NH_3-N/g VSS d. The range for r_N values obtained in present study is comparable with other various wastewater types reported in literature as shown in Table 4.9. Typically, nitrification rate was observed to rise with increase in OLR which could be responsible in gradual increase in ammonia-nitrogen and organic matter concentration. Although, parameters such as DO and alkalinity addition to maintain neutral pH were sustained at average of 4.5±0.3 of 7.6±0.1 as discussed in Section 4.4.4.

Table 4.9: Specific nitrification rates reported for different wastewater

Type of wastewater	r_N (mg NH_3-N/g VSS d)	Reference
Dairy farm wastewater	2-12	[132]
Synthetic wastewater	13-31	[132]
Domestic sewage	30-180	[29]
Piggery wastewater	12-17	[320]
Beverage wastewater	6-11	This study

A statistical analysis (ANOVA) was conducted on the effluent clarifier ammonia nitrogen results obtained from the experimental data. At 95% confidence level, the result has shown significant difference ($P<0.05$) between the mean values of the concentration at respective HRT's (Table 4.10), where the results indicated that mean

value for HRT with flow rate of 45 L/d was lower than the mean values of other flow rates having 70 L/d and 100 L/d. Hence, HRT with a flow rate of 45 L/d achieved better performance of nitrification performance compared to HRT with flow rates of 70 L/d and 100 L/d.

Table 4.10: Phase II-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on Ammonia-Nitrogen performance.

```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.
>> [p,tbl,stats] = anova1(AMMONIA1)

p =

  1.5543e-015


tbl =

    'Source'      'SS'         'df'    'MS'         'F'            'Prob>F'
    'Columns'     [7.3607]     [ 2]    [3.6803]     [193.1278]     [1.5543e-015]
    'Error'       [0.4574]     [24]    [0.0191]           []               []
    'Total'       [7.8180]     [26]          []           []               []


stats =

    gnames: [3x1 char]
         n: [9 9 9]
    source: 'anova1'
     means: [1.5933 2.2978 2.8700]
        df: 24
         s: 0.1380

>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -0.8670   -0.7044   -0.5419
    1.0000    3.0000   -1.4392   -1.2767   -1.1142
    2.0000    3.0000   -0.7347   -0.5722   -0.4097


m =

    1.5933    0.0460
    2.2978    0.0460
    2.8700    0.0460
```

4.5.3.2 Phase II results for removal of nitrate-nitrogen

For denitrification process to occur, nitrate, COD, and denitrifying bacteria are required in an anoxic environment with pH values ranging between 6 and 9. Most denitrifying bacteria are heterotrophic and require an organic carbon source for cell growth and nitrate reduction [321]. The characteristics of the added carbon source have been found to have major effects on important parameters of the denitrification process such as the denitrification rate, kinetics, and COD demand [322]. The biological denitrification of wastewater depends on a number of factors, such as the highest nitrate concentration that the microorganisms can tolerate and availability of organic carbon [323].

Combining anoxic and aerobic units with nitrate recycle has been commonly used for nitrogen removal in full-scale wastewater treatment plants [324]. In an anoxic reactor receiving wastewater influent and recycled flow with nitrate, the denitrifiers use the organic carbon in the influent as the electron donor and the nitrate as the electron acceptor, releasing nitrogen into the atmosphere. This process may remove up to 80 % of the NO_3-N when a 400% recycle rate is used [84].

Nitrate-nitrogen was sampled from the influent, E-ANX-C, E-AER-C (IR) and E-CLR. Nitrates into ANX-C were determined based on combined flow from influent, IR and RAS flow nitrate-nitrogen concentrations according to Equation 3.27. Denitrification process in the ANX-C was achieved through recycling of the nitrates formed in the AER-C during the nitrification process. Denitrification performance was assessed based on denitrification efficiency (η_D) and specific denitrification rates Calculations to determine r_D was realized from obtaining the ratio of the difference of nitrate mass entering and leaving the ANX-C and MLVSS mass (g) in ANX-C expressed as mg NO_3-N/g VSS d according to Equation 3.28 and Equation 3.30 (Table 3.7, Section 3.6). Detailed analysis is contained in Appendix E (Table E1.1).

In this study, influent wastewater was utilized as a biodegradable carbon source for denitrification. Hence, ratio of COD consumed to nitrate reduced ($\Delta COD/\Delta NO_3$-N) was estimated for each steady state of the OLR. Profile for nitrate-nitrogen concentration in the E-AER-C and E-CLR are shown in Figure 4.37. Figure 4.38 shows trend for nitrates into ANX-C, denitrified E-ANX-C and η_D.

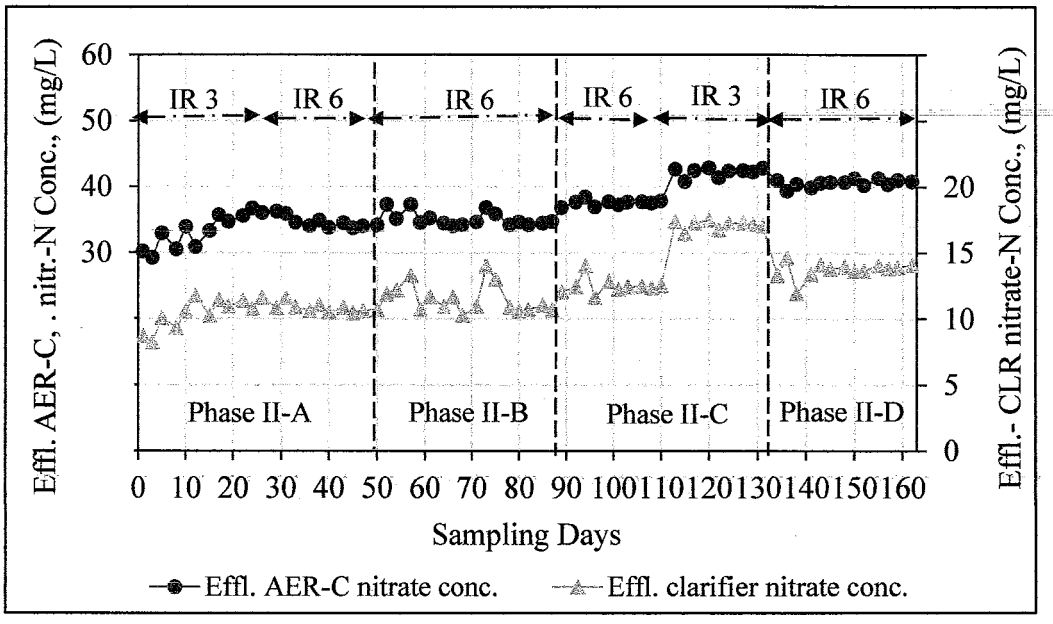


Figure 4.37: Aeration chamber and Effl.- Clarifier nitrate Conc. vs Sampling Days

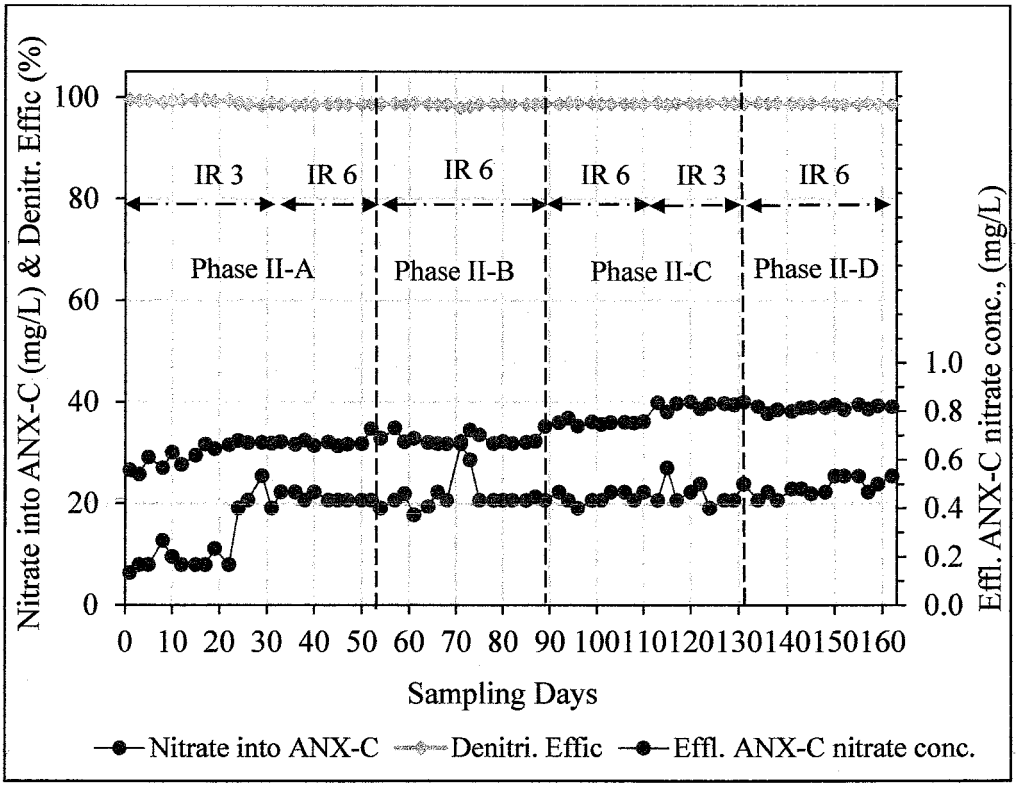


Figure 4.38: Nitrate into Anoxic and Effl-Anoxic nitrate Conc. vs Sampling Days

Influent nitrate-nitrogen concentration throughout phase II was observed to have average value and standard deviation of 0.4±0.1 mg/L. This is expected considering nitrate from food and beverage industry is only generated from oxidation of ammonia-nitrogen by nitrifiers. As can be seen from Figure 4.37, it can be observed that the accumulation of nitrate-nitrogen in the AER-C increases with a rise in the OLR from phase II-A through phase II-D. Interpretation of steady state results according to Figure 4.37 and Figure 4.38 as shown in Figure 4.39.

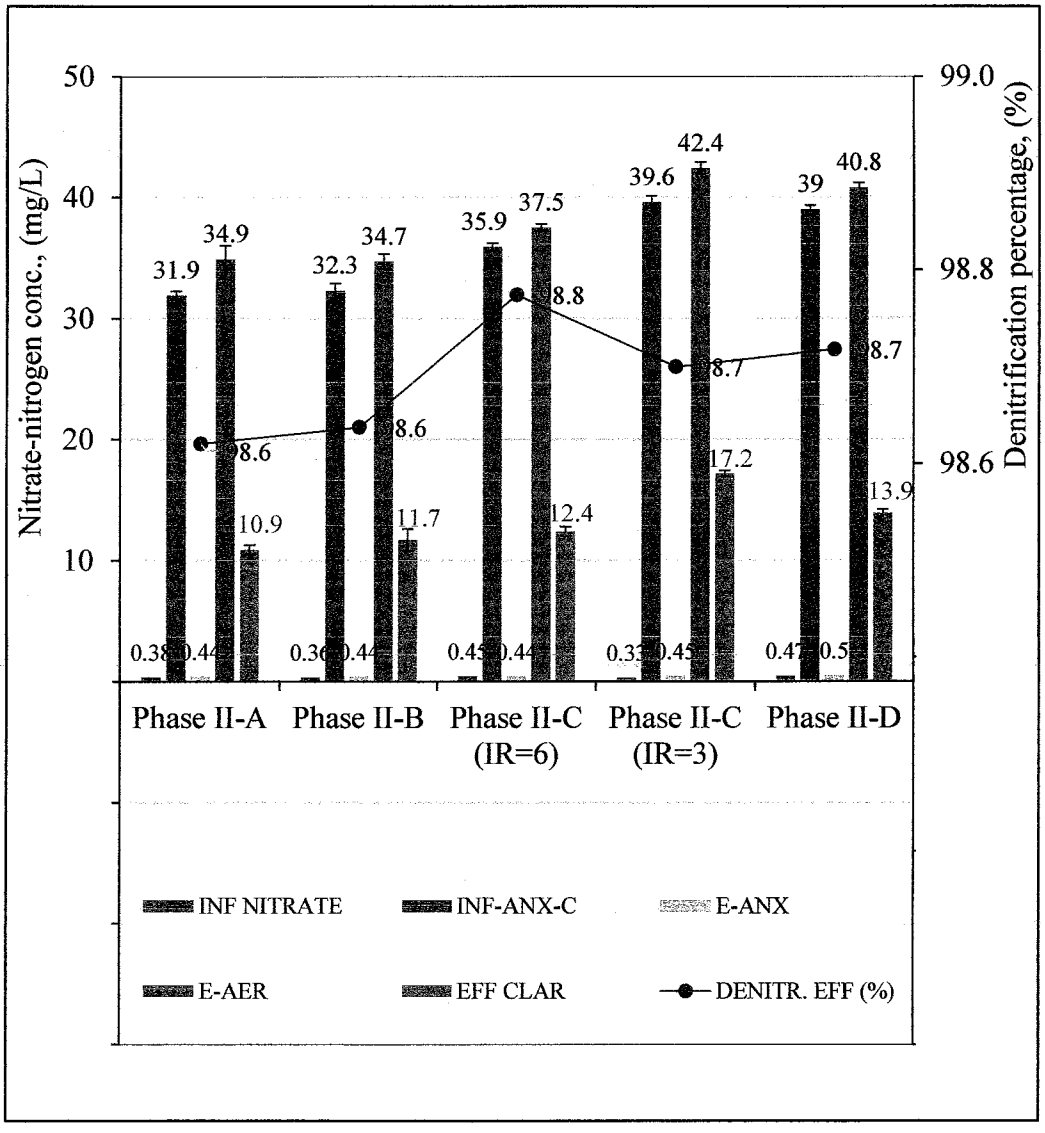


Figure 4.39: Steady state results for Infl, Infl.-Anoxic, Effl.-Anoxic, Effl-Aeration, and Effl.-Clarifier nitrate Conc. vs Phases

In phase II-A, the operation was performed with an influent flow rate of 45 L/d and internal recycle ratio of 3 (135 L/d). IR ratio of 3 was considered to stabilize the reactor in anticipation to allow for slow nitrifier growth. Initially, the E-CLR nitrate at start up was very low which could be due to anoxic might denitrify all the limited E-AER-C nitrate concentration. However, probably due to the non-wasting of biomass and maintaining high AER-C HRT of 66.7 hours, nitrate nitrogen concentration from E-AER-C on day 17 was observed to have average value of 35.7 mg/L, with equivalent E-CLR average values of 11.5 mg/L. Steady state was observed between day 19 to day 50 with average value and standard deviation for E-CLR nitrate-nitrogen observed as 10.9±0.4 mg/L. Equivalent influent and E-AER-C nitrate concentration were observed to be 0.38±0.1 mg/L and 34.9±1.1 mg/L, respectively. Based on combined flow, influent ANX-C, and E-ANX-C nitrate-nitrogen concentrations were observed to be 31.9±0.36 mg/L and 0.44±0.04 mg/L, respectively. The nitrogen loading rate was determined as 0.25±0.5 g NO_3-N/d. Specific denitrification rate (r_D) was observed to have average value and standard deviation of 94.8±17.9 mg NO_3-N/g VSS d. In the ANX-C, average value and standard deviation for specific COD removal rate of 0.304±0.08 mg COD/mg VSS d was observed, where equivalent $\Delta COD/\Delta NO_3$-N ratio was observed to be 22.4±5.9. The denitrification percentage (η_D) based on efficiency derived from ANX-C was observed to be 98.6±0.11 %.

In phase II-B, the operation was done with IR ratio of 6 corresponding to IR flow of 270 L/d. Aerobic digestion started on day 50, where from days 52 to 57 an observed accumulation in nitrate-nitrogen concentration in E-AER-C and E-CLR was observed. The peak nitrate nitrogen concentration was observed on day 57 to have E-AER-C and E-CLR average values of 37.3 mg/L and 13.3 mg/L, respectively. Increase in nitrate nitrogen observed could be due to EAD resulting from oxidation of ammonia nitrogen from digested sludge. Between days 59 to 87 steady state was observed with an average value and standard deviation for E-CLR nitrate-nitrogen concentration of 11.7±0.9 mg/L. Corresponding influent and AER-C nitrate nitrogen concentration were observed as 0.36±0.07 mg/L and 34.7±0.6 mg/L, respectively. Nitrate nitrogen into ANX-C and E-ANX-C were observed to be 32.3±0.6 mg/L and 0.44±0.01 mg/L, respectively. Nitrate loading rate into the ANX-C during the steady state was observed to be 0.27±0.06 g NO_3-N/m^3 d. The η_D percentage based on η_D

derived from ANX-C performance was observed to be 98.7±0.03 %. The r_D was observed as 95.9±2.2 mg NO_3-N/g VSS d. In the ANX-C, where the specific COD removal rate of 0.28±0.08 mg COD/mg. VSS. d was determined, the ANX-C $\Delta COD/\Delta NO_3$-N ratio was observed to be 22.5±6.4. On the quality perspective, there was an operational problem due to malfunction of the ANX-C mixer between days 73 to 75 was observed, with detected high effluent nitrate observed having average values in the E-CLR recorded as 14 mg/L and 10.9 mg/L, respectively. Accumulation due to nitrate concentration was also observed in similar days 73 and 75, with the AER-C nitrate concentration rising to values of 36.9 mg/L on day 73 and 35.9 mg/L on day 75. Although, mixing was resorted and achieved manually pending rectification on day 75.

In phase II-C, the flow rate was increased to 70 L/d for the operation and two regimes of IR ratio of 6 between days 89 to 113, and IR ratio of 3 between days 113 to 131 were operated to determine the impact on the denitrification efficiency. At steady state between days 101 to 131, overall average value and standard deviation for η_D of 98.8±0.05 % was observed. The influent nitrate, E-AER-C, and E-CLR were observed as 0.36±0.1mg/L, 39.9±2.4 mg/L and 14.8±2.4 mg/L, respectively. Nitrate loading rate into the ANX-C was observed to be 0.38±0.06 g NO_3-N/m^3d. For the ANX-C denitrification, the concentration of nitrates into ANX-C and E-ANX-C were observed as 37.6±1.84 mg/L and 0.46±0.04 mg/L, respectively. The r_N, specific COD removal rate in ANX-C and $\Delta COD/\Delta NO_3$-N ratio were observed to have average values and standard deviation of 145±3.5 mg NO_3-N/g VSS d, 0.35±0.13 mg COD/mg. VSS d and 17.1±5.9, respectively. Distinctively, based on the individual performances, the IR ratio of 6 (IR flow of 420 L/d) was observed to have average value and standard deviation for η_D of 98.8 ±0.1 %, with E-CLR average nitrate concentration value and standard deviation of 12.4±0.4 mg/L and effluent AER-C nitrate concentration of 37.5±0.3 mg/L. Based on combined flow, I-ANX-C and E-ANX-C nitrate concentration were observed to have average and standard deviation values of 35.9±0.3 mg/L and 0.44±0.03 mg/L, respectively. On the other hand, IR ratio of 3 corresponding to IR flow of 210 L/d was observed to achieve η_D of 98.7±0.4 %. However, with an observed rise in E-AER-C and E-CLR nitrate concentration, which was observed with an average value and standard deviation of 42.4±0.5 mg/L and

17.2±0.23 mg/L, respectively. This phenomenon could be due to nitrate concentration build up in the AER-C without attempts to denitrify it. The combined nitrate into the ANX-C and E-ANX-C was observed to have average and standard deviation values of 39.6±0.5 mg/L and 0.45±0.04 mg/L, respectively. The equivalent influent was observed to be 0.33 ±0.12 mg/L as an average value and standard deviation. Comparing denitrification performance between IR ratio of 6 and IR ratio of 3, 28.1 % higher efficiency was derived from IR ratio of 6 with difference in E-CLR nitrate concentration observed as 4.84 mg/L.

Phase II-D was operated with a flow rate of 100 L/d and IR ratio of 6, corresponding to flow rate of 600 L/d and RAS flow of 50 L/d. Between days 101 to 131, steady state was observed, and the nitrate loading was observed to have average value and standard deviation of 0.57±0.1 g NO_3-N/m^3d. For the steady state, E-CLR nitrate-nitrogen concentration of 13.9±0.2 mg/L was observed. This corresponds to influent and E-AER-C nitrate nitrogen concentration observed with an average value and standard deviation as 0.47±0.1 mg/L and 40.8±0.4 mg/L, respectively. Nitrate nitrogen into ANX-C and E-ANX-C were observed to be 39±0.34 mg/L and 0.5±0.03 mg/L, respectively. η_D percentage based on ANX-C performance was observed as 98.7±0.09%. r_D was observed as 158±17 mg NO_3-N/g VSS d. In the ANX-C, specific COD removal rate and $\Delta COD/\Delta NO_3$-N ratio in ANX-C were observed with average value and standard deviation of 0.29±0.12 mg COD/mg VSS d and 13.4±5.1, respectively.

The nature of carbon source has intense effect on r_D, where higher rates are easily achieved with biodegradable forms [173, 325]. Various studies have reported r_D values for some carbon sources such as 56 and 146 mg NO_3-N/g VSS d for methanol [326-328], 65 mg NO_3-N/g VSS d for glucose [329], 16 to 603 mg NO_3-N/g VSS d for acetate [330], and 18 mg NO_3-N/g VSS d for starch [326]. The r_D achieved in this research with beverage wastewater in addition to recycle sludge as carbon source for denitrification, were relatively within the range compared to other reported values of 60 to 170 for some beverage wastewater obtained from other studies [30, 331].

Four scenarios were tested for the nitrate nitogen concentration using statistical analysis (ANOVA) and multiple comparison ad hoc tests from results obtained in experimental data.

a. First scenerio: This scenario considered effect of HRT and OLR on effluent clarifier nitrate concentration based on related conditions (flow rates of 45, 70 and 100 L/d with constant IR ratio 6 operated).

b. Second scenario: This was based on varied IR ratio of 6 and 3 operated during phase II-C, with constant influent flow rate of 70 L/d, which was to observe effect of HRT on denitrification performance for the E-ANX-C nitrate concentration.

c. Third scenario: This scenerio was to check effect of HRT on performance of effluent-anoxic chamber nitrate-nitrogen with constant IR ratio of 6, using three regimes of flow rates (45, 70 and 100 L/d).

d. Fourth scenario: This scenerio tested denitrification performance in phase II-C, with constant HRT and varied IR ratio of 6 and 3, from effluent anoxic chamber nitrate-nitrogen concentration.

The result for first scenario indicated that at 95% confidence level, there exist a significant difference ($P<0.05$) for effluent clarifier nitrate nitrogen concentration, based on increase in OLR, reduction of HRT, and constant IR ratio of 6 (Table 4.11). Consequently, result for multiple comparison has shown that mean value for effluent clarifier nitrate with IR ratio of 6 and flow rate of 45 L/d had lower mean nitrate concentration compared to other effluent clarifier nitrate concentrate with flow rates having 70 L/d and 100 L/d, respectively. The increase in effluent clarifier nitrate nitrogen concentration could be due to increase in nitrate loading rate from oxidation of organic matter in aeration chamber, which likely resulted active decrease in contact time for denitrification process performance.

Table 4.11: Statistical Analysis (ANOVA) and Multiple Comparison for Effect of Hydraulic Retention Time on Effluent Clarifier nitrate-nitrogen performance

```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.
>> [p,tbl,stats] = anova1(NITRATE1)

p =

  1.3117e-006


tbl =

    'Source'     'SS'          'df'    'MS'          'F'           'Prob>F'
    'Columns'    [23.5200]     [ 2]    [11.7600]     [38.1405]     [1.3117e-006]
    'Error'      [ 4.6250]     [15]    [ 0.3083]           []               []
    'Total'      [28.1450]     [17]           []           []               []


stats =

    gnames: [3x1 char]
         n: [6 6 6]
    source: 'anova1'
     means: [11.1500 12.5500 13.9500]
        df: 15
         s: 0.5553

>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -2.2327   -1.4000   -0.5673
    1.0000    3.0000   -3.6327   -2.8000   -1.9673
    2.0000    3.0000   -2.2327   -1.4000   -0.5673


m =

   11.1500    0.2267
   12.5500    0.2267
   13.9500    0.2267
```

Second scenario was operated with constant flow rate of 70 L/d and varied IR ratio of 6 and 3. Result has shown that at 95% confidence level, effluent clarifier nitrate concentration was significant ($P<0.05$), which was operated with relatively constant OLR and HRT (Table 4.12). Subsequently, on multiple comparison results indicated that the mean value for effluent clarifier nitrate concentration with IR ratio of 6 having flow rate of 45 L/d, was lower than the mean value for effluent nitrate concentration operated with IR ratio of 3 at 70 L/d. Hence, it can be concluded that effluent clarifier nitrate concentration operated with IR ratio of 6 achieved better denitrification performance compared to effluent clarifier nitrate concentation operated with IR ratio of 3.

Similar reason can be referred where increase in effluent clarifier nitrate nitrogen concentration could be due to increase in the nitrate rich mixed liquor transfer rate into anoxic chamber from oxidation of organic matter in aeration chamber, which might lead to effective reduction in contact time to perform denitrification activity, as well increase in nitrate nitrogen capture from effluent aeration chamber which could escape into the clarifier, where some part will be released in effluent clarifier, while part would be returned to anoxic chamber via RAS for denitrification and recharge of biomass in the activated sludge system. Thus, it was observed that effluent clarifier nitrate concentration decreases with decrease in IR ratio.

Table 4.12: Statistical Analysis (ANOVA) and Multiple Comparison for Effect of Hydraulic Retention Time and Internal Recycle of Nitrate in Effluent clarifier

```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.

>> [p,tbl,stats] = anoval(nitrate2)

p =

  1.4178e-012


tbl =

  Columns 1 through 5

    'Source'      'SS'          'df'    'MS'          'F'
    'Columns'     [80.1607]     [ 1]    [80.1607]     [872.2150]
    'Error'       [ 1.1029]     [12]    [ 0.0919]           []
    'Total'       [81.2636]     [13]           []           []

  Column 6

    'Prob>F'
    [1.4178e-012]
                  []
                  []


stats =

    gnames: [2x1 char]
         n: [7 7]
    source: 'anoval'
     means: [12.4286 17.2143]
        df: 12
         s: 0.3032
```

```
>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -5.1388   -4.7857   -4.4326


m =

   12.4286    0.1146
   17.2143    0.1146
```

Results for third scenario have shown significant difference ($p<0.05$) in effluent-ANX nitrate nitrogen concentration for the ANOVA tested at 95 % confidence interval (Table 4.13). The multiple comparison stats further confirmed that the highest HRT equivalent to lowest OLR operated with flow rate of 45 L/d was observed with lowest E-ANX-C nitrate concentration compared to other flow rates of 70 and 100 L/d operated at constant IR ratio of 6.

Table 4.13: Statistical Analysis (ANOVA) and multiple comparism for Effect of Hydraulic Retention Time on performance of Effluent-Anoxic nitrate-nitrogen

```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.
>> [p,tbl,stats] = anova1(Anoval_nitrate1)

p =

    0.0046


tbl =

    'Source'      'SS'          'df'     'MS'                'F'          'Prob>F'
    'Columns'     [0.0167]      [ 2]     [      0.0083]     [8.7178]     [0.0046]
    'Error'       [0.0115]      [12]     [9.5667e-004]           []           []
    'Total'       [0.0282]      [14]                   []           []           []


stats =

    gnames: [3x1 char]
         n: [5 5 5]
    source: 'anova1'
     means: [0.4340 0.4520 0.5120]
        df: 12
         s: 0.0309

>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -0.0702   -0.0180    0.0342
    1.0000    3.0000   -0.1302   -0.0780   -0.0258
    2.0000    3.0000   -0.1122   -0.0600   -0.0078


m =

    0.4340    0.0138
    0.4520    0.0138
    0.5120    0.0138
```

Therefore, it can be concluded that HRT had effect on effect on effluent-ANX nitrate concentration, however, not to large extent which could be due to increase in ANX-C MLVSS concentration to enhance denitrification activity.

Results for fourth scenario were tested for ANOVA and multiple comparison at 95 % confidence interval. Performance based on constant HRT and OLR and varied IR ratio was assessed. It was observed that there was no significant difference (P>0.05) for E-ANX-C nitrate nitrogen concentation, and based on multiple comparison of mean values for E-ANX-C nitrate nitrogen concentration, similar E-ANX-C nitrate nitrogen concentration for IR ratio of 6 and 3 were obtained (Table 4.14).

Table 4.14: Statistical Analysis (ANOVA) and Multiple Comparison for Effect of Same Hydraulic Retention Time and varied Internal recycle ratio on Effluent Anoxic nitrate-nitrogen Performance

```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.
>> [p,tbl,stats] = anova1(nitrate3)

p =

    1


tbl =

    'Source'    'SS'          'df'    'MS'          'F'    'Prob>F'
    'Columns'   [     0]      [ 1]    [     0]      [0]    [     1]
    'Error'     [0.0343]      [12]    [0.0029]       []           []
    'Total'     [0.0343]      [13]          []       []           []


stats =

    gnames: [2x1 char]
         n: [7 7]
    source: 'anova1'
     means: [0.4429 0.4429]
        df: 12
         s: 0.0535
```

The condition with similar E-ANX-C nitrate nitrogen concentration could be due to system operation with constant HRT and OLR, where denitrification was consistent at the operated IR ratios of 6 and 3, but effluent clarifier nitrate nitrogen concentration was observed to vary, which could be due to varied IR ratio that could affect nitrate nitrogen concentration in aeration chamber and effluent clarifier.

4.5.3.3 Phase II results for removal of Total nitrogen and Total kjeldahl nitrogen

Samples were obtained from influent and E-CLR of the reactor to determine TN and TKN. The objective was to compare TN with contribution of its respective nitrogen forms in effluent such TKN, ammonia nitrogen, nitrate nitrogen and nitrite. Quality checks were performed on the effluent sample for nitrite concentration to ensure there was no nitrite accumulation in the reactor. The influent and effluent data for nitrogen forms were used to assess the composition of nitrogen forms at each steady state. Total nitrogen (TN) comprises both organic and inorganic (ammonium, nitrate and nitrite) forms [82].

Average daily concentrations and standard deviations for TKN, ammonia nitrogen, nitrite, nitrate and TN in influent and effluent clarifier are plotted in Figure 4.40 and steady state data for the duration is shown in Figure 4.41. The accurate nitrogen mass balance could not be established because nitrogen was not measured from sludge. However, the data is to establish the composition of nitrogen forms both in influent and effluent.

From the time series plot in Figure 4.40 and values for the steady state in Figure 4.41, it can be seen that the influent TN and TKN concentration for the duration in phase II ranges between 68.4 mg/L to 88.4 mg/L, and 67.7 mg/L to 87.7 mg/L, respectively. In influent, it can be observed that TN concentration were similar to TKN concentration for phase II-A through phase II-D. The difference was seen to come from the influent nitrate concentration, which ranges between 0.3 mg/L to 0.4 mg/L. Presence of nitrate in influent could probably be due to transportation of the wastewater in pipeline or storage in balance tank, where in transition and during mixing microorganisms could likely be induced to tranform some metabolic activities.

Equivalent TN and TKN concentrations in the effluent clarifier ranges between 10.6 to 19.7 mg/L, and 1.4 to 3.0 mg/L respectively. The removal efficiency of TN between 76.8 to 83.7 % was achieved (Figure 4.41), where effluent nitrate concentration was between 59.4 to 66.9 % of the TN concentration. However, cocnsistent removal of nitrate was achieved from E-ANX-C above 98 %. The trend can be seen from graph of influent and E-CLR TN and TKN concentration for phase II as shown in Figure 4.40.

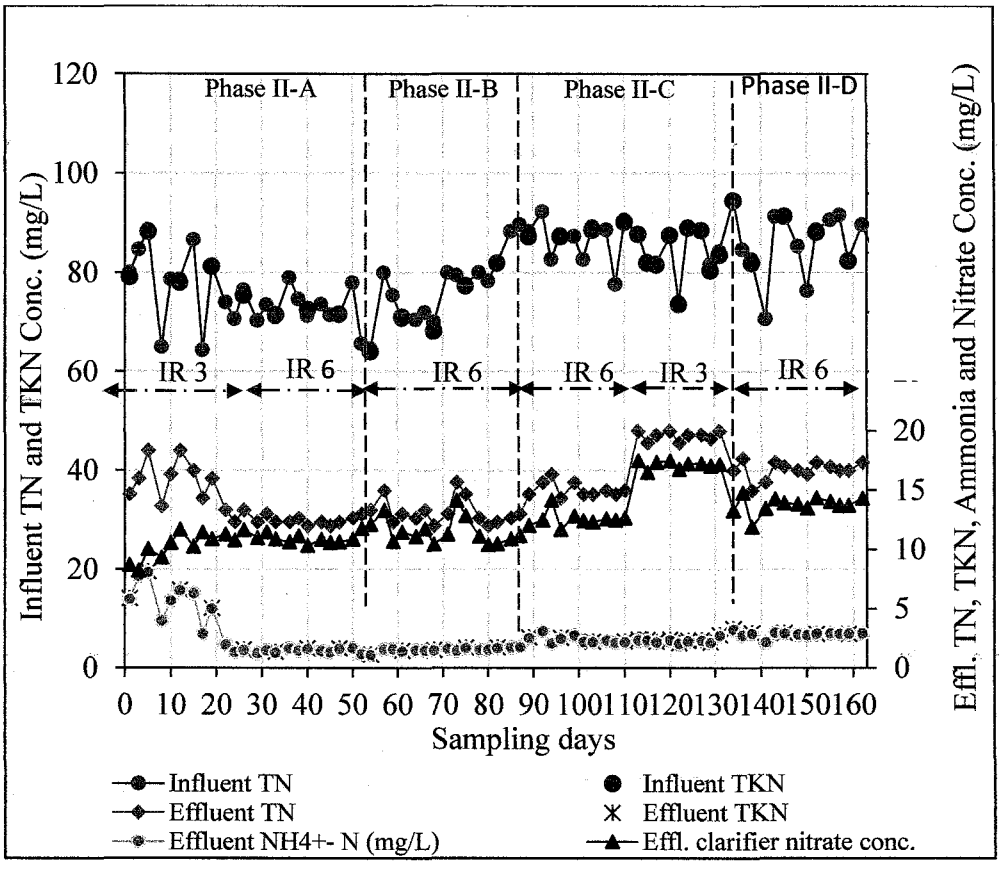


Figure 4.40: Profile for Total nitrogen conc. forms in infl. and eff. vs Sampl. Days

Throughout phase II, it was observed that influent organic nitrogen assessed from TKN and ammonia-nitrogen constituted average percentage value for TKN of 37 %, which corresponds to average value and standard deviation 29.3±3.8 mg/L, while influent TKN constitutes an average percentage of 63.4 % with an average value and standard deviation observed as 80±9.4 mg/L. The influent ammonia nitrogen was observed to have average and standard deviation o 50.7±5.7 mg/L. Conversely, effluent TN and TKN were observed to exhibit a different pattern, where it was observed that effluent TN had more content of nitrate nitrogen of up to 85 %. The equivalent effluent

TN and nitrate nitrogen were observed to have concentration with average value and standard deviation of 15±3.1 mg/L and 13±2.6 mg/L, respectively.

It was observed that the effluent concentration of TKN and ammonia nitrogen were also similar. The average value and standard deviation for TKN and ammonia nitrogen were observed to be 2.1±0.7 mg/L and 2.1±0.6 mg/L, respectively. Effluent organic nitrogen was observed to be approximately 3 % of the TKN concentration. The effluent organic nitrogen was observed to have average value and standard deviation of 0.1 mg/L. Effluent nitrite was measured and concentration throughout the experimental period was observed have average value and standard deviation of 0.06±0.02 mg/L.

As can be seen in phase II-C as shown in Figure 4.40, the effect of IR can be seen on TN and nitrate removal but not on ammonia nitrogen removal. Removal of ammonia nitrogen ranges between 98.6 to 98.8 %, as such nitrification efficiency was observedd to be consistent. When IR was set to 6 between day 89 to day 110, effluent TN and nitrate nitrogen concentrations were observed with average concentration value and standard deviation of 14.4±0.3 mg/L and 12±0.3 mg/L. Between day 113 to day 131 when IR was set to 3, effluent TN and nitrate nitrogen concentrations were observed with average concentration value and standard deviation of 19.7±0.3 and 17±0.1 mg/L, respectively. Hence, it was observed the IR of 6 with recycle flow of 420 L/d has provided 26 % and 30 % additional performance in TN and nitrate-nitrogen removal efficiency, than it was observed during operation with IR of 3 (IR flow of 210 L/d). There was no significant impact seen on the effect of IR flow on ammonia nitrogen removal. For the ANX-C and AER-C HRT operated, effluent ammonia nitrogen concentration was observed with IR of 6 (IR flow of 420 L/d) with an average value and standard deviation of 2.4±0.2 mg/L. Consequently, when IR of 3 was operated effluent clarifier ammonia nitrogen concentration was observed with an average concentration and standard deviation of 2.39±0.3 mg/L. The equivalent influent ammonia nitrogen concentration for IR 6 and IR 3 were observed to be 55.8 mg/L and 53.9 mg/L, respectively. Detailed nitrogen inventory for the steady state and operational conditions in both influent and effluent clarifier is shown in Figure 4.41.

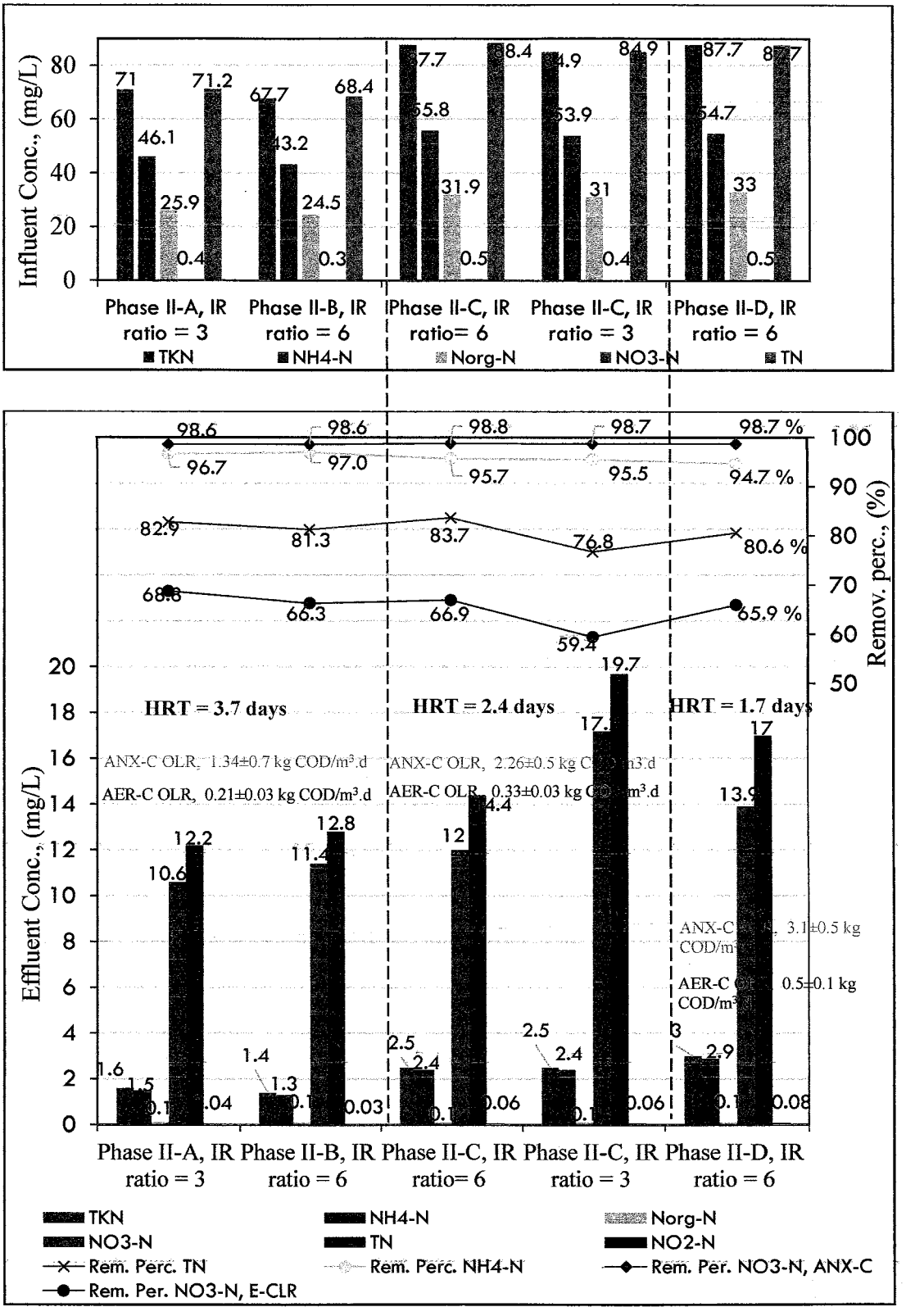


Figure 4.41: Steady state results for Infl. and Effl. Nitrogen Inventory and Removal Efficiency vs Phases

4.5.3.4 Phase II summary of operation, control parameters and performance data

Phase II-A was achieved between days 1 to 50. The influent flow rate operated was 45 L/d, RAS flow of 35 L/d (RAS ratio = 0.8) and IRQ of 135 L/d (IR ratio = 3). This was the start-up phase, where long HRT of 5.89 days was operated in expectation to avoid biomass washout. The RAS ratio of 0.8 was operated. Steady state was observed between days 57 to 87. Average and standard deviation values for MLSS concentrations in ANX-C, AER-C and RAS were 3565±229, 3113±102, and 6142±246 mg/L, respectively. The operation was performed in a suspended growth mode during the entire period of the study.

Phase II-B was achieved between days 52 to 87. The operation was carried out with an influent flow rate of 45 L/d, RAS flow of 270 L/d (RAS ratio = 0.8) and IRQ of 270 L/d (IR ratio = 6), to include start-up of sludge digestion monitoring. i-SGBR system SRT was 39.7±1.7 days and aerobic digester was operated for 10 days SRT. Steady state was observed for the operation between days 57 to 87, with average and standard deviation values for MLSS concentration in ANX-C, AER-C, and RAS as 3454±68 mg/L, 3191±143 mg/L, and 6209±199 mg/L, respectively. HRT for ANX-C, AER-C and AD-C were 21.0 hrs, 66.7 hrs, and 24 hrs, respectively. The average OLR operated for ANX-C and AER-C during were 1.34±0.68 kg COD/m^3.d and 0.21±0.03 kg COD/m^3.d. F/M ratio in AER-C was 0.04±0.01 kg COD/kg MLVSS d.

Phase II-C was operated with an influent flow rate of 70 L/d, RAS flow of 35 L/d (RAS ratio = 0.5), two IRQ regimes of 420 L/d (IR ratio =6) and 210 L/d (IR ratio = 3). Sludge digestion was included and collectively achieved between days 89 to 131. i-SGBR system SRT was 38.9±1.7 days and aerobic digester was operated at 10 days SRT. i-SGBR system HRT operated was 1.43 days. The corresponding HRT operated for ANX-C, AER-C and AD-C were 13.7 hrs, 42.8 hrs, and 24 hrs, respectively. Steady state for the operation was observed between day 57 to day 87. Average OLR operated for ANX-C and AER-C were 2.26±0.46 kg COD/m^3 d and 0.33±0.03 kg COD/m^3 d, respectively. The AER-C F/M ratio was 0.05±0.01 kg COD/kg MLVSS d. The average and standard deviation values for MLSS concentration in ANX-C, AER-C, and RAS were 3691±73 mg/L, 3479±136 mg/L, and 7097±127 mg/L, respectively.

Phase II-D operation was achieved between days 134 to 162 with an influent flow rate of 100 L/d, RAS flow rate of 50 L/d (RAS ratio = 0.5) and IRQ of 600 L/d (IR ratio = 6) to include sludge digestion. i-SGBR system SRT was 38.3±3.2 days and aerobic digester was operated at 10 days SRT, and i-SGBR system HRT operated was 1.25 days. The corresponding ANX-C, AER-C and AD-C HRT operated were 9.6 hrs, 30 hrs, and 24 hrs, respectively. Steady state for the operation was observed between days 143 to 152. Average ANX-C and AER-C OLR operated were 3.05±0.52 kg COD/m^3.d and 0.50±0.06 kg COD/m^3.d, respectively. The AER-C F/M ratio was 0.06±0.01 kg COD/kg MLVSS d. The average and standard deviation values for MLSS concentration in ANX-C, AER-C, and RAS were 5101±232 mg/L, 3745±96 mg/L, and 12,252±647 mg/L, respectively. Between day 155 to day 162, biomass was build up, where AER-C MLSS concentration of 5,473 mg/L was achieved on day 159. The summary for actual operational conditions discussed for phase II are presented in Table 4.15, summary of pH, temperature and DO operational parameters are contained in Table 4.16, and summary of data for COD, sCOD, BOD_5, and TSS are contained in Table 4.17 and summary of data for TN, TKN, NH_3-N, NO_3-N in Table 4.18.

Table 4.15: Phase II results for i-SGBR operational parameters

Phase	II			
Experimental sub-phase	II-A (Start-up period)	II-B	II-C	II-D
Experiment period (days)	1-50	52-87	89-131	134-162
Flow rate, Q_{INF}, (L/d)	45		70	100
Hydraulic retention time Θ_{ANX-C}, HRT (hours)	21.0		13.7	9.6
Hydraulic retention time Θ_{AER-C}, HRT (hours)	66.7		42.8	30
Hydraulic retention time Θ_{I-SGBR}, HRT (days)	5.89		1.78	1.25
Hydraulic retention time, aerobic digester, Θ_{AD-C}	-	24 hrs		
Hydraulic retention time, Clarifier, Θ_{CLAR} (days)	2.22		1.43	1.00
Organic loading rate, (OLR), ANX-C, (kg COD/m^3.d)	1.34±0.68		2.26±0.46	3.05±0.52
Organic loading rate, (OLR), AER-C, (kg COD/m^3.d)	0.21±0.03		0.33±0.03	0.50±0.06
F/M ratio for AER-C, kg COD/m^3. d	0.04±0.01		0.05±0.01	0.06±0.01
Solids retention time Θ_c, SRT (days)	40.8±1.4	39.7±1.7	38.9±1.7	38.3±3.2
Solids retention time Θ_c, SRT_{AD-C} (days)	-	10		
Internal recycle ratio, IR	3	6	6 & 3 - effects of IR on denitri	6
Internal recycle flow, Q_{INF} *IR, (L/d)	135	270	420 & 210	600
Vol. Pumped in each cycle (12 cycles), (L)	11	23	35 | 18	50
Pumping duration, (minutes)	1.0	2.0	2.5 | 1.5	4.0
SVI, (mL/g)	157±6.6	148±5.4	132±4.7	92±17
MLSS concentration, ANX-C, mg/L	3449±54	3511±88	3691±73	4918±92
MLVSS concentration, ANX-C, mg/L	2672±93	2996±273	3061±103	4322±93
MLSS concentration, AER-C, mg/L	3113±102	3191±143	3479±136	3745±96
MLVSS concentration, AER-C, mg/L	2711±103	2770±151	2958±135	3212±69
MLSS concentration in CLR, mg/L	6142±246	6299±133	7113±190	12,517±272
MLVSS concentration, CLR, mg/L	-	4798±131	5990±305	10,049±604
Working RAS ratio, Ri = [X/(Xr-X)]	0.8	0.8	0.5	0.5
Total RAS flow (R_{Total}) = (R_i) * Q_{INF}, (L/d)	35	35	35	50

Table 4.16: Phase II results for pH, DO, and temperature

Experimental phases	Operational Period (days)	Parameter	Measured values			
			Chamber	Max.	Av. ±SD	Min.
II-A	24-50	pH	INF	6.8	5.89±0.7	4.2
			ANX-C	7.8	7.5±0.1	7.3
			AER-C	7.8	7.6±0.1	7.2
			AD-C	-	-	-
		DO (mg/L)	ANX-C	0.28	0.23±0.02	0.18
			AER-C	5.75	4.48±0.3	3.76
			AD-C	-	-	-
		Temp. (°C)	AER-C	32.1	29.0±1.3	27.3
II-B	50-87	pH	INF	6.6	5.7±0.8	4.2
			ANX-C	7.6	7.4±0.1	7.3
			AER-C	7.7	7.6±0.1	7.3
			AD-C	7.3	7.1±0.1	6.8
		DO (mg/L)	ANX-C	0.28	0.23±0.03	0.18
			AER-C	5.11	0.18±0.80	0.15
			AD-C	9.33	8.28±0.53	6.93
		Temp. (°C)	AER-C	33.3	29.2±0.5	27.3
II-C	87-131	pH	INF	6.6	6.0±0.5	4.7
			ANX-C	7.7	7.4±0.1	7.3
			AER-C	7.6	7.5±0.1	7.2
			AD-C	7.3	7.1±0.1	6.9
		DO (mg/L)	ANX-C	0.27	0.24±0.02	0.21
			AER-C	5.02	4.51±0.28	4.09
			AD-C	9.11	7.99±0.51	7.08
		Temp. (°C)	AER-C	32.5	30.0±1.1	28.6
II-D	131-162	pH	INF	6.8	6.3±0.4	5.3
			ANX-C	7.6	7.5±0.1	7.3
			AER-C	7.7	7.6±0.1	7.4
			AD-C	7.3	7.1±0.1	7.0
		DO (mg/L)	ANX-C	0.27	0.24±0.02	0.21
			AER-C	4.92	4.42±0.27	4.06
			AD-C	8.99	8.47±0.38	7.80
		Temp. (°C)	AER-C	32.7	29.2±1.2	27.9

Table 4.17: Phase II results summary of steady state performance data for COD, sCOD, and TSS

Phase II	SSP	SP	**COD (mg/L)**	**sCOD (mg/L)**	**BOD_5 (mg/L)**	**TSS (mg/L)**		**Ratio**	
			Av.±SD	Av.±SD	Av.±SD	Av.±SD		COD/BOD_5	COD/TSS
II-A	24-50	**INF**	1273±219	481±52	804±122	653±92		0.6	2.0
II-B	75-87		1276±224	459±67	760±69	664±100		0.6	1.9
II-C	99-131		1169±349	520±68	759±62	660±54		0.7	1.8
II-D	143-162		1300±167	257±11	851±46	666±54		0.7	2.0
II-A	24-50	**E-ANX**	571±34	231±7					
II-B	75-87		543±18	252±14					
II-C	99-131		680±32	257±11					
II-D	143-162		723±15	268±17					
II-A	24-50	**E-AER**		28±2					
II-B	75-87			35±6					
II-C	99-131			44±2					
II-D	143-162			50±2					
II-A	24-50	**RAS**		24±2					
II-B	75-87			29±4					
II-C	99-131			35±2					
II-D	143-162			44±4					
II-A	24-50	**E-CLR**	27±2	17±2	8±2	22±3		0.3	1.2
II-B	75-87		35±2.9	23±4	6±2	26±2.3		0.2	1.4
II-C	99-131		46±3	28±2	16±4	34±3.9		0.3	1.4
II-D	143-162		69±2	34±2	18±1	48±3		0.3	1.4

Notes: SSP = Steady state period, SP = Sampling point, INF = Influent, E-ANX = Effluent anoxic chamber, E-AER = Effluent aeration chamber, RAS = Return activated sludge, E-CLR = Effluent clarifier, COD = Total chemical oxygen demand, sCOD = Soluble chemical oxygen demand, BOD_5 = Biochemical oxygen demand, and TSS =Total suspended solids

Table 4.18: Phase II results summary of steady state performance data for TKN, TN, Ammonia and Nitrate

Phase II	SSP	SP	TN (mg/L)	TKN (mg/L)	NH_3-N (mg/L)	NO_3-N (mg/L)	Ratio	
			Av.±SD	Av.±SD	Av.±SD	Av.±SD	COD/N	COD/NO_3
II-A	24-50	INF	71.2±0.2	71.1±0.1	46.1±1.6	0.38±0.1	17.4	
II-B	75-87		68.4±3.2	67.7±3.5	48.5±3.6	0.36±0.07	12.5	
II-C	99-131		88.4±0.8	87.7±0.8	53.5±3.5	0.45±0.1	21.4	
II-D	143-162		87.7±4.6	87.3±4.6	54.7±3.2	0.47±0.1	5.6	
II-A	24-50	I-ANX				31.9±0.36		22.4±5.9
II-B	75-87					32.3±0.6		22.5±6.4
II-C	99-131					36±0.3		17.1±5.9
II-D	143-162					39±0.3		13.4±5.1
II-A	24-50	E-ANX				0.44±0.04		
II-B	75-87					0.44±0.01		
II-C	99-131					0.44±0.03		
II-D	143-162					0.5±0.03		
II-A	24-50	E-AER				34.9±1.1		
II-B	75-87					34.7±0.6		
II-C	99-131					37.5±0.3		
II-D	143-162					40.8±0.4		
II-A	24-50	E-CLR	12.2±0.6	1.57±0.2	1.5±0.1	10.9±0.4		
II-B	75-87		12.8±0.8	1.39±0.2	1.6±0.1	11.7±0.9		
II-C	99-131		14.4±0.3	2.47±0.2	2.2±0.1	12.4±0.4		
II-D	143-162		17±0.4	2.91±0.1	2.9±0.1	13.9±0.3		

Notes: INF =Influent, I-ANX = Influent anoxic chamber, E-ANX-C = Efluent anoxic chamber, E-AER = Effluent aeration, E-CLR = Effluent clarifier

4.6 Phase III results for system operation and control parameters

In Phase III operation was conducted between day 162 to 278. Five consecutive flow rates were operated corresponding to ANX-C and AER-C HRT's according to Table 4.19. The objective was to evaluate the influence of HRT on reactor performance. Operation and control parameters monitored for i-SGBR system include MLSS, MLVSS, SVI, pH, DO, temperature, HRT, OLR, F/M, SRT, IRQ, and RAS flow.

Table 4.19: Phase III i-SGBR system operational parameter

Phase	**III**				
Experimental stage	III-A	III-B	III-C	III-D	III-E
Experimental period (days)	164-185	185-208	208-232	232-255	255-278
Flow rate, Q_{INF}, (L/d)	100	110	120	135	150
Hydraulic retention time Θ_{ANX-C}, HRT (hours)	9.6	8.7	8.0	7.1	6.4
Hydraulic retention time Θ_{AER-C}, HRT (hours)	30	27.3	25.0	22.2	20.0
Hydraulic retention time Θ_{I-SGBR}, HRT (days)	2.65	2.41	2.21	1.96	1.77
Hydraulic retention time, Clarifier, Θ_{CLAR} (days)	1.00	0.91	0.83	0.74	0.67

In phase III-A, the experiment was carried out between day 164 to 185, phase III-B was achieved between day 187 to 208, phase III-C experiment was achieved between day 211 to 232, phase III-D was achieved between day 234 to 255, and phase III-E was achieved between day 257 to 278. These phases have operational flow rates corresponding to influent flow rates of 100, 110, 120, 135 and 150 L/d, respectively. Equivalent ANX-C and AER-C HRT for influent flow rate of 100, 110, 120, 135 and 150 L/d were 9.6 hrs, 8.7, 8.0, 7.1, 6.4, and 30, 27.3, 25, 22.2 and 20 hrs, respectively. AD-C HRT of 1.00 day was maintained for entire phase.

4.6.1 Phase III results for MLSS, MLVSS, and SVI

The results for MLSS, MLVSS, and SVI for phase III operation are presented in this section. ANX-C was monitored for MLSS and MLVSS to evaluate the parameters such as specific denitrification rates and specific COD removal rates. AER-C was monitored

for MLSS, MLVSS and SVI to evaluate parameters such as specific COD removal rates, SRT control, F/M ratio, and SVI sludge settling properties. RAS MLSS concentration was monitored from measured and validated with observed values evaluated from AER-C MLSS and SVI according to Section 3.1.7.9.

4.6.1.1 Phase III results for anoxic chamber MLSS and MLVSS concentration

The changes in the biomass concentration for ANX-C at different OLR and HRT from phase III-A through phase III-E is shown in Figure 4.42. General increase in solids accumulation was observed from phase III-A to phase III-E, which could be due to increase in OLR in the reactor system. ANX-C receives combined biomass from RAS flow, IR flow and possible decay resulting from the anoxic process.

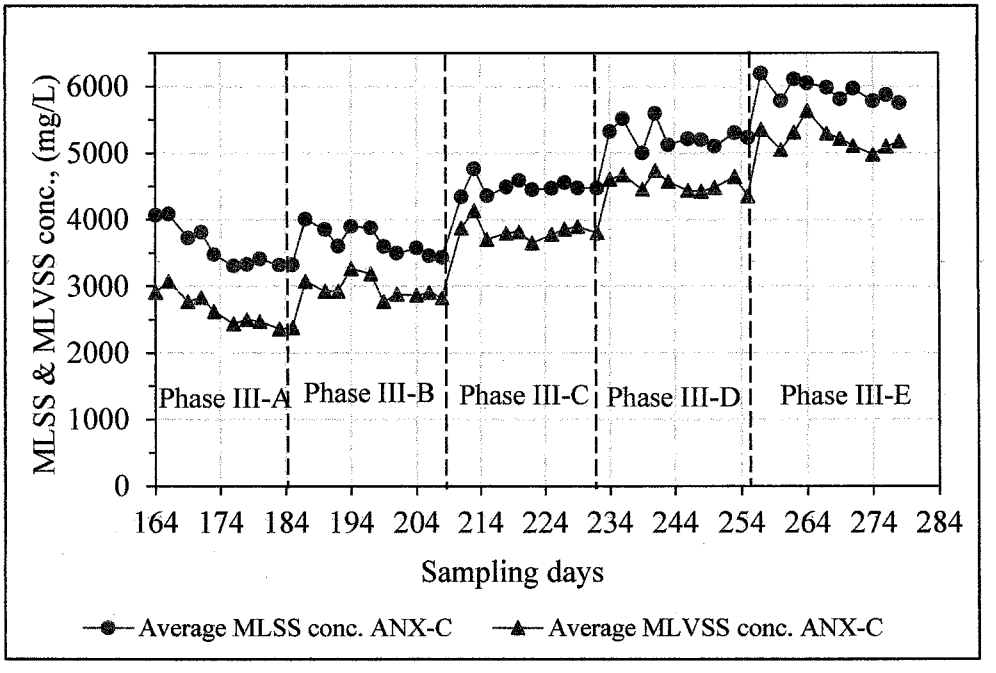


Figure 4.42: Anoxic chamber Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids Conc. vs Sampling Days

In phase III-A, steady state was observed between day 173 to 185, where the ANX-C MLSS and MLVSS concentrations were observed with average values and

standard deviation of 3362±69 and 2466±94 mg/L, respectively. This gives ANX-C MLVSS/MLSS ratio of 0.73.

In phase III-B operation, steady state was observed between day 199 to day 208, where ANX-C MLSS and MLVSS average value and standard deviation were observed as 3514±72 mg/L and 2854±54 mg/L, respectively. This gives ANX-C MLVSS/MLSS ratio of 0.81.

In phase III-C operation. The average and standard deviations values for ANX-C MLSS and MLVSS at steady state observed between day 222 to day 232 were observed as 4490±42 mg/L and 3800±94 mg/L, respectively. This gives corresponding ANX-C MLVSS/MLSS ratio of 0.84.

In phase III-D operation, steady state was observed between day 246 to 255, where ANX-C MLSS and MLVSS concentration were observed as 5215±74 mg/L and 4473±109 mg/L, respectively. This gives ANX-C MLVSS/MLVSS ratio of 0.86.

In phase III-E operation, steady state was observed between day 267 to 278, where ANX-C MLSS and MLVSS concentration were observed as 5841±90 mg/L and 5119±64 mg/L, respectively. This gives ANX-C MLVSS/MLVSS ratio of 0.87.

4.6.1.2 Phase III results for aeration chamber MLSS, MLVSS and SVI profile

The trend in changes for the biomass concentration for AER-C MLSS and MLVSS is shown in Figure 4.43, and profile for SVI monitored in the AER-C is shown in Figure 4.43. MLSS control in the AER-C was achieved through wasting of sludge.

In phase III-A, experiment was conducted between day 164 to 185, where AER-C MLSS/MLVSS steady state was observed between day 173 to day 185, with AER-C MLSS and MLVSS concentration observed to be 5057±27 and 3021±78 mg/L, respectively. This gives AER-C MLVSS/MLSS ratio of 0.6. Ratio was observed to be low, although, MLVSS/MLSS ratio for extended aeration was given in the range of 0.6 to 0.75 [332].

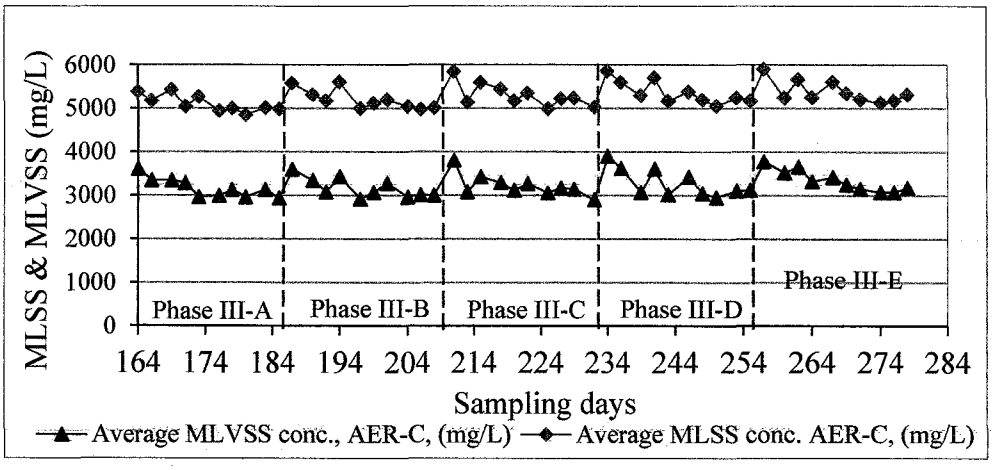


Figure 4.43: Aeration chamber Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids Conc. vs Sampling Days

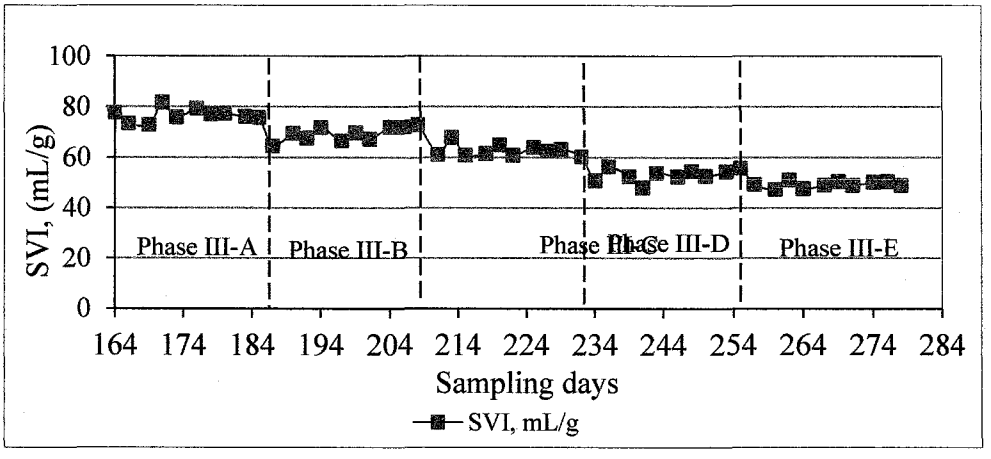


Figure 4.44: Sludge Volume Index profile vs Sampling Days

The low ratio could also be due to endogenous process in the AER-C from probable reduced substrate offer to the microorganisms. Equivalent SVI values were observed as an average value and standard deviation of 76±1.3 mL/g.

Phase III-B operation was achieved between 185 to day 208. Between day 199 to day 208, steady state was observed for AER-C MLSS/MLVSS with an average value and standard deviation of AER-C MLSS and MLVSS concentration of 5070±89 mg/L and 3086±126 mg/L, respectively. This gives AER-C MLVSS/MLSS ratio value of 0.6. SVI for phase III-B as shown in Figure 4.44 was observed with an average value and standard deviation of 148±5.4 mL/g at steady state.

In phase III-C, the experiment was conducted between day 208 to day 232 with the observed steady state for AER-C MLSS and MLVSS concentration observed on day 22. The average and standard deviations values of AER-C MLSS and MLVSS at steady state were observed between day 222 to day 232. The observed values at steady state for the AER-C MLSS and MLVSS were observed as 5167±135 and 3104±140 mg/L, respectively. This gives MLVSS/MLSS ratio of 0.6. SVI was observed to be stable in phase III-C as shown in Figure 4.37, with a downward decrease in the SVI value from phase III-B to average and standard deviation values of 62±1.8 mL/g.

Phase III-D operation was achieved between day 246 to day 255 with the observed steady state for AER-C MLSS and MLVSS between day 246 to day 255. The average value and standard deviation of AER-C MLSS and MLVSS concentration were observed as 5202±109 mg/L and 3178±176 mg/L, respectively. This gives AER-C MLVSS/MLVSS concentration of 0.6. Prevailing SVI values at steady state were observed with average and standard deviation values of 55±1.28 mL/g.

Phase III-E operation was achieved between 255 to day 278, with observed AER-C MLSS and MLVSS steady state observed between day 269 to day 278. The observed AER-C MLSS and MLVSS had average values and standard deviation of 5299±172 mg/L and 3227±99 mg/L, respectively. This gives AER-C MLVSS/MLSS ratio value of 0.6. SVI for phase III-E as shown in Figure 4.42 was observed with an average value and standard deviation of 50±1.6 mL/g at steady state.

4.6.1.3 Phase III results for relationship between experimental and predicted RAS concentration

The experimental MLSS concentration for RAS (X_r) observed was compared with predicted MLSS, which was measured from AER-C MLSS and SVI according to Equation 3.18 and Equation 3.19 in order to validate the experimental MLSS concentration observed. The time series profile for the experimental and measured MLSS is shown in Figure 4.45 for phase III.

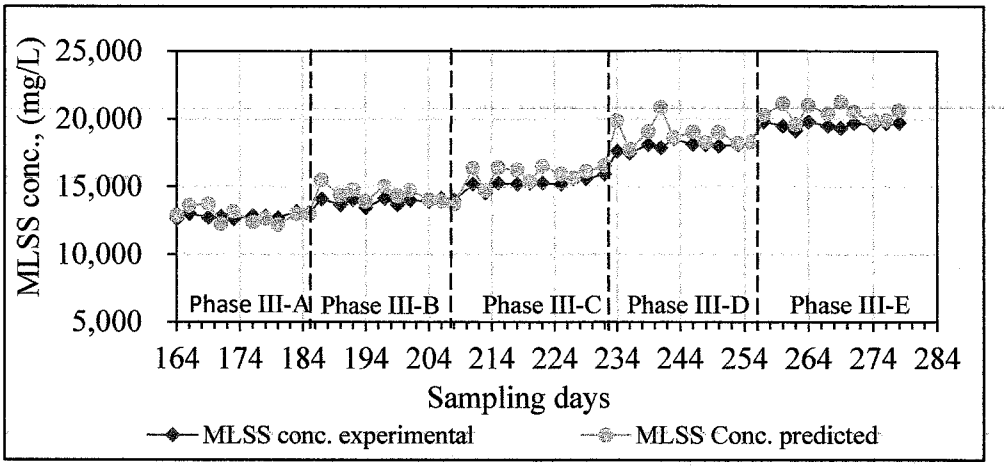


Figure 4.45: Experimental and predicted MLSS concentration vs Sample. Days

To establish the difference, ANOVA analysis was carried out and the means between the experimental and predicted MLSS concentrations. At 95 % confidence interval, ($p>0.05$) it was observed that there was no any significant difference between measured and observed MLSS concentration (Table 4.20). This indicates a good level of accuracy between measured MLSS by experiment and predicted MLSS obtained from calculations with available AER-C MLSS and SVI values.

Table 4.20: Phase III Statistical Analysis (ANOVA) for experimental and predicted RAS concentration

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MLSS conc. experimental	50	796650	15933	6521065
MLSS conc. predicted	50	826613	16532.26	8325539

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8977802	1	8977802	1.21	0.27	3.93
Within Groups	7.27E+08	98	7423302			
Total	7.36E+08	99				

4.6.2 Phase III results for aerobic digestion and mass balance

AD-C was operated as batch digester system from phase III-A through phase III-E, where it was fed once daily and decanted once daily based on 10 days SRT. Volume of 7.5 L was fed into the AD-C from underflow and 7.5 L was displaced on daily basis. The purpose of operating AD-C unit was to reduce sludge production as the system operates. Hence, endogenous metabolism process was the mechanism adopted to reduce sludge wastage, in which the AD-C was fed with raw sludge. Efficiency of the AD-C was evaluated for solids reduction (MLSS and MLVSS). Mass of digested sludge from EAD was compared with the mass of the wasting rate. The solids balance was established on MLSS around AD-C boundary because sludge wasting was according to MLSS. Details of data for solids determination in the experiments from phase III-A through phase III-E is highlighted in (Table C1.2 in Appendix C).

Figure 4.46 through Figure 4.51 show sequence for the daily variation in the levels of IAD and EAD MLSS and MLVSS concentration (mg/L), IAD and EAD mass of MLSS and MLVSS (mg/d), and MLSS and MLVSS mass balance within the AD-C boundary.

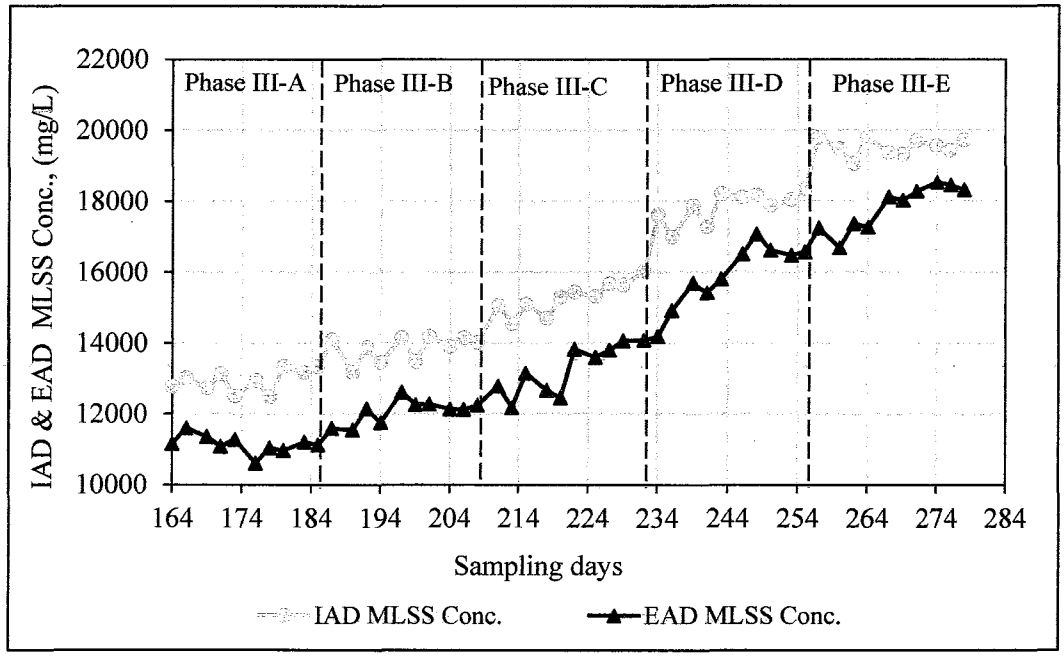


Figure 4.46: Influent and Effluent Aerobic Digester Mixed Liquor Suspended Solids Conc. vs Sampling Days

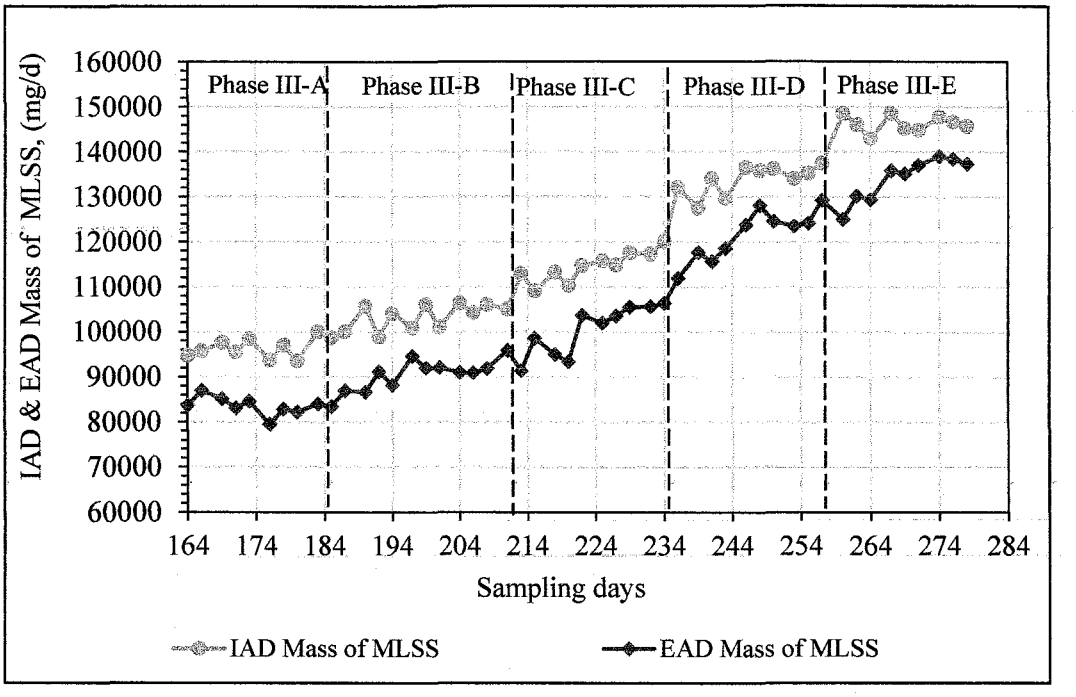


Figure 4.47: Influent and Efluent mass of Aerobic Digester Mixed Liquor Suspended Solids vs Sampling Days

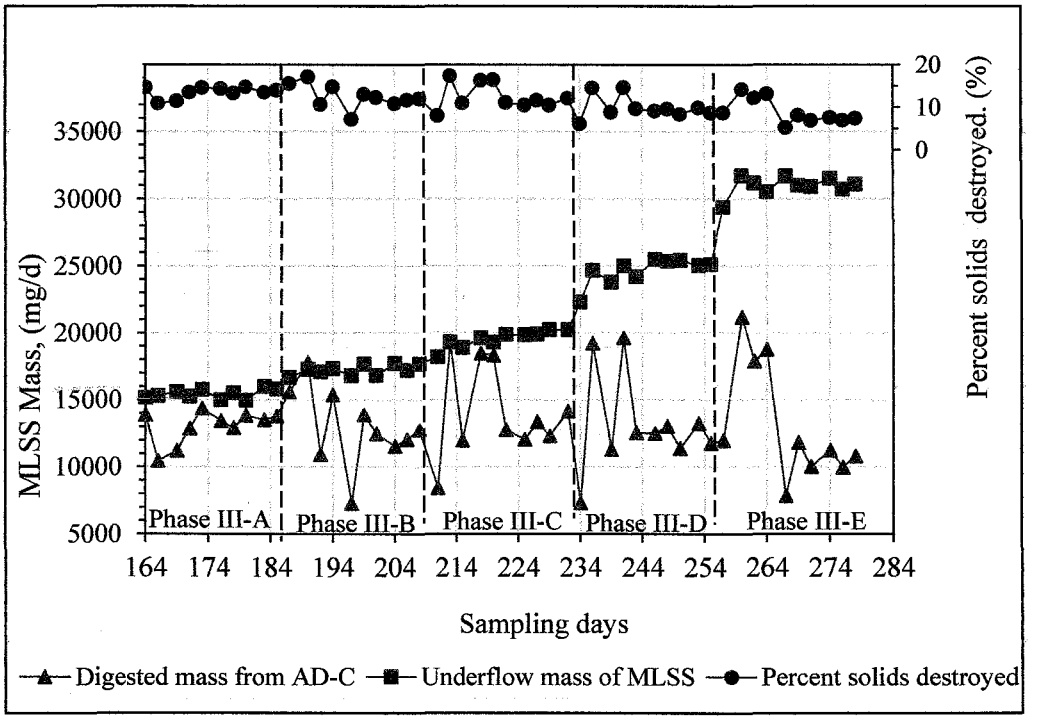


Figure 4.48: Aerobic Digester Mixed Liquor Suspended Solids Mass balance and Degadation Efficiency vs Sampling Days

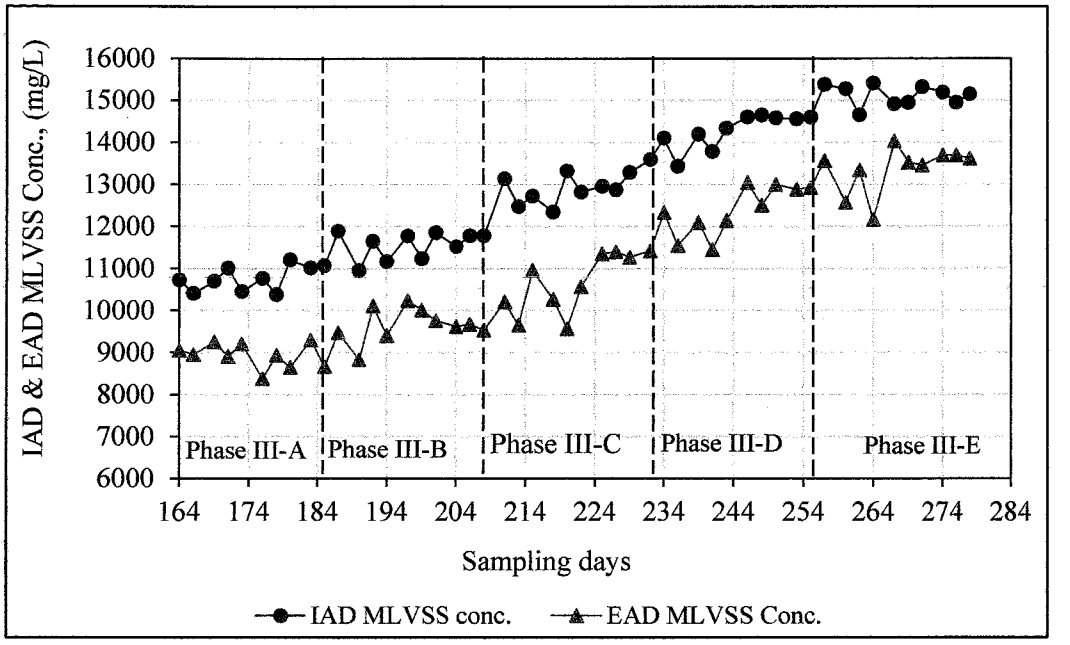


Figure 4.49: Influent and Effluent Aerobic Digester Mixed Liquor Volatile Suspended Solids Conc. vs Sampling Days

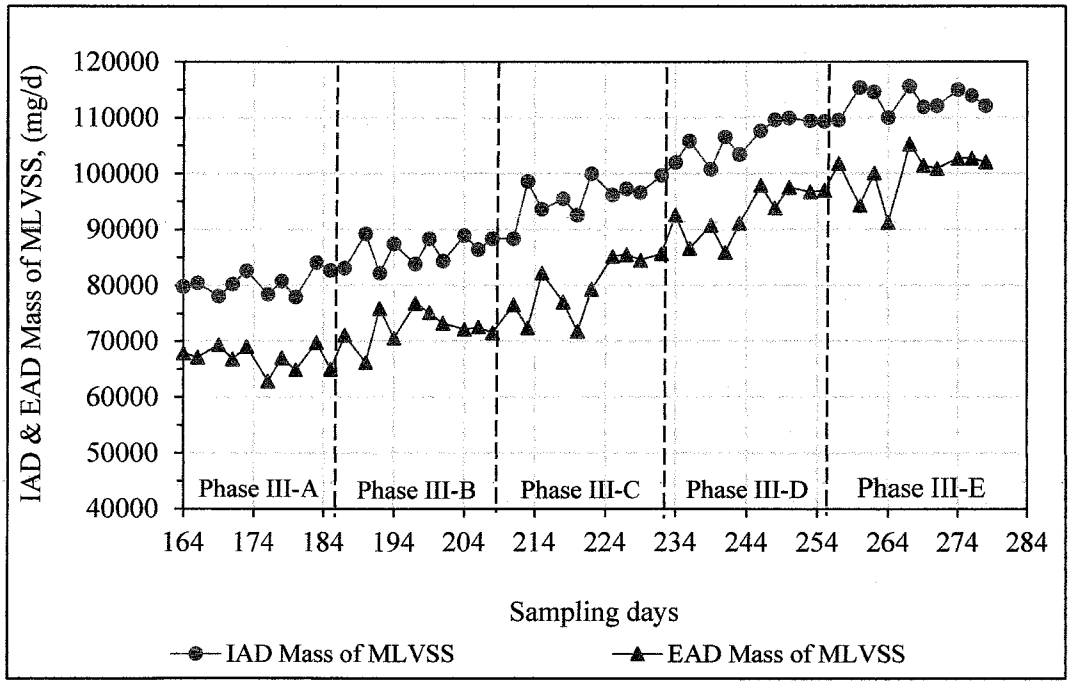


Figure 4.50: Influent and Effluent mass of Aerobic Digester Mixed Liquor Volatile Suspended Solids vs Sampling Days

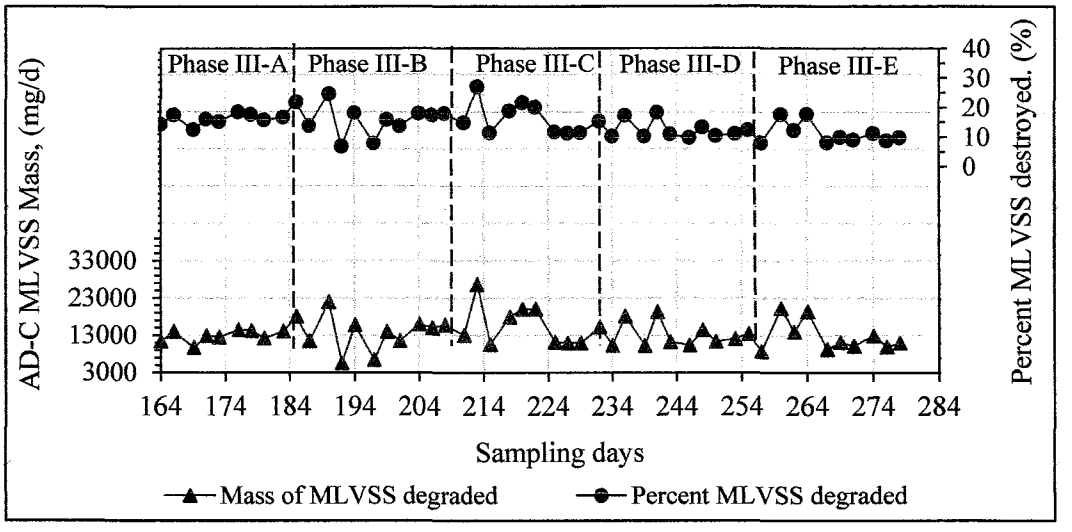


Figure 4.51: AD-C MLVSS mass degraded and percent degraded vs Sampl. Days

In phase III-A aerobic digester operation, steady state was observed between day 178 to 185 in EAD MLSS and MLVSS concentrations (see Figure 4.49 and Figure 4.50). The average and standard deviations values for IAD and EAD MLSS, and IAD and EAD MLVSS values were observed as (13,055±360 and 10,992±232 mg/L), and (10,893±257 and 8,790±348 mg/L), respectively. This gives equivalent IAD and EAD MLVSS and MLSS ratio of 0.83 and 0.80, respectively. The ratio of MLVSS/MLSS in EAD was observed to be lower than the IAD ratio, which could suggest that the system may have under gone endogenous metabolism process, where more MLVSS were degraded than MLSS. The equivalent IAD and EAD mass of MLSS were observed to be 96,655±2,914 and 82,440±1,745 mg/d. For the MLVSS, the IAD mass of MLVSS and EAD mass of MLVSS were observed as 80,765±2495 and 65,925±2610 mg/d. The MLSS and MLVSS degraded observed in AD-C were 14,265±1029 and 14,640±1986 mg/d, respectively. The corresponding percentage degradation of the MLSS and MLVSS were observed as 14.3 and 18.6 %, respectively.

In phase III-B, steady state operation was observed between day 201 to 208. Observed IAD and EAD MLSS concentrations were 13,917±294 and 12,208±71 mg/L, respectively. This gives IAD and EAD MLVSS/MLSS ratio of 0.83 and 0.79, respectively. Equivalent MLVSS concentrations for the IAD and EAD were observed

with average and standard deviation values of 11,609±275 and 9,724±183 mg/L. Consequently, the IAD and EAD mass of MLSS and IAD and EAD mass of MLVSS were observed as (104,580±2331 and 91,565±535 mg/d) and (87,070±2064 and 72,930±1377 mg/d), respectively. The degraded MLSS and MLVSS in the AD-C were observed with average mass and standard deviation of 13,615±1380 mg/d and 14,290±1788 mg/d, respectively. Percentage degradation of MLSS and MLVSS in the AD-C were observed to be 12.7 % and 17.1 %, respectively.

Phase III-C steady state operation was observed between day 225 to 232. Average and standard deviation values for IAD and EAD MLSS concentration, and IAD and EAD MLVSS concentrations were observed as (15,622±270 and 13,877±202 mg/L), and (13, 113±326 and 11, 206±357 mg/L), respectively. This gives IAD MLVSS/MLSS and EAD MLVSS/MLSS ratios of 0.84 and 0.80, respectively. The corresponding average and standard deviation values for IAD and EAD mass of MLSS were observed as 116,070±1,331 and 104,075±1,521 mg/d. On the other hand, IAD mass of MLVSS and EAD mass of MLVSS were observed to be 97,940±1,764 and 84,050±2,675 mg/d. MLSS and MLVSS degraded in the AD-C were observed with average and standard deviation value of 12,995±742 and 13,740±2094 mg/d, respectively. Consequent percentage degradation of MLSS and MLVSS in the AD-C were observed as 11.1 % and 14.3 %, respectively.

Phase III-D operation had its steady state observed between day 248 to 255. The IAD and EAD MLSS concentrations were observed to be 18,107±169 mg/L and 16,648±245 mg/L, respectively. This gives an average of IAD MLVSS/MLSS ratio and EAD MLVSS/MLSS ratio of 0.81 and 0.78, respectively. Corresponding average values and standard deviation for MLVSS concentrations observed in IAD and EAD were 14,606±113 mg/L and 12,881±214 mg/L. Subsequently, observed mass of MLSS for IAD and EAD and mass of MLVSS for IAD and EAD were (135,465±1105 and 124,860±1841 mg/d) and (109,160±1064 and 96,605±1,607 mg/d), respectively. MLSS and MLVSS degraded in the AD-C were observed with average mass and standard deviation of 11,955±1145 and 12,405±1,614 mg/d, respectively. The degradation efficiency expressed as percentage in the AD-C were observed as 8.6 % and 10.7 % for MLSS and MLVSS, respectively.

In phase III-E operation of aerobic digestion, steady state was observed between day 269 to day 278 was observed. The average values and standard deviations for IAD and EAD MLSS concentrations, and IAD and EAD MLVSS concentrations were observed as (19,475±236 and 18,232±189 mg/L), and (15,021±197 and 13,658±185 mg/L), respectively. This gives corresponding IAD and EAD MLVSS and MLSS ratio of 0.77 and 0.74, respectively. The equivalent IAD and EAD mass of MLSS were observed as 145,525±1,432 and 136,740±1,414 mg/d. MLVSS mass for IAD and mass of MLVSS for EAD were observed as 112,460±1443 and 102,435±1,384 mg/d. The observed degraded MLSS and MLVSS in the AD-C were 10,635±789 and 11,075±907 mg/d, respectively. The corresponding percentage degradation of the MLSS and MLVSS were observed to be 7.3 % and 9.8 %, respectively.

Qualitative observations of all the sludge samples used as IAD were observed to be brownish in color, while all the digested solids were observed as light brown. There was no detectable and noticeable odor observed in the AD-C. This could possibly be due to the continuous aeration and exposure of the AD-C.

4.6.3 Phase III results of heterotrophic plate count and population of microfauna

Determination of bacteria colonies was carried out and presented as heterotrophic plate count. The population of microfauna were determined for the samples in the aeration chamber, and microrganisms counted in optical microscope for protozoa and metazoa. The results for the bacteria colony forming units and microscopic observations is presented in this Section.

4.6.3.1 Phase III results for heterotrophic plate count

The viable bacterial population in the aeration chamber was determined for MLSS concentrations of 5,000, 5,100, 5,200, 5,300 and 5,500 mg/L, respectively. The method adopted followed 3M Petrifilm Aqua Plate Testing procedure, which involves serial dilution of sludge samples, where 1 mL diluted sludge (10^{-6}) was pipetted and directly plated to prepared nutrient media. The samples were incubated for 48 hours at 35°C.

After the incubation was completed, colonies were counted. The microorganisms counted are presented as colony forming units (cfu/mL) and results observed presented according to Table 4.21 and Figure 4.52. The microbial growth appearing on each individual media was counted and enumerated as HPC (cfu/mL).

Table 4.21: Phase III results for heterotrophic plate count of various sludge conc.

Aeration tank MLSS, (mg/L)	Heterotrophic plate count, (cfu/mL) x 10^7			
	Sample 1	Sample 2	Sample 3	Average count
5,000	6.3	6.1	6.7	6.37
5100	6.9	7.5	7.3	7.23
5200	8.0	7.7	8.2	7.97
5,300	8.8	9.0	8.6	8.80
5,500	9.4	9.8	9.6	9.60

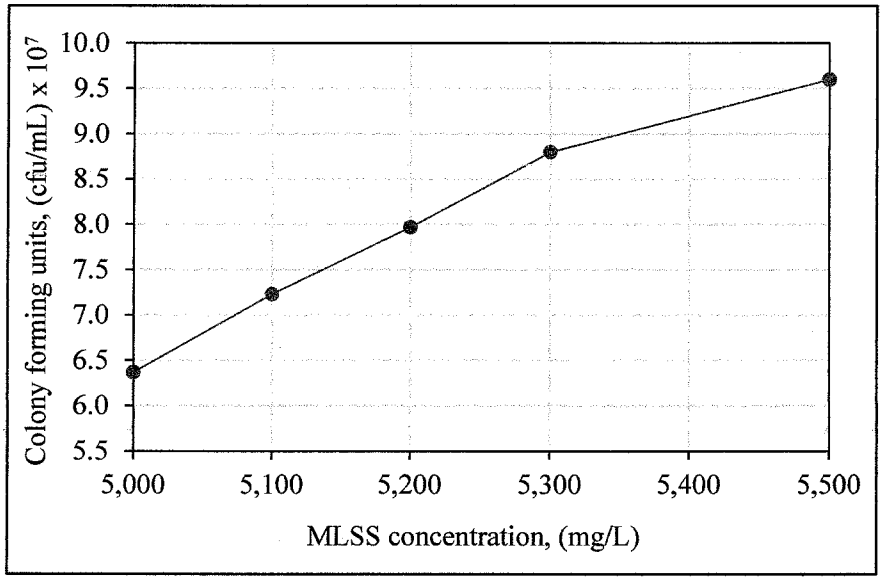


Figure 4.52: Variation of plate counts with aeration chamber sludge

As can be seen from Figure 4.52, the relationship between the observed HPC and MLSS concentration progressively ascends with increasing MLSS concentration. The observed MLSS of 5, 500 mg/L resulted in higher observed HPC count of 9.60 x 10^7 cfu/mL. Hence, minimum and maximum MLSS concentrations observed with MLSS of 5,000 mg/L and 5,500 mg/L resulted HPC average counts of 6.37x10^7 and 9.60x10^7 cfu/mL, respectively. The difference between HPC with MLSS 5,000 and 5,100 mg/L was observed as 11.9 %, and difference between the HPC with MLSS 5,200

and 5,300 mg/L was observed as 9.3 %. The difference between HPC with MLSS concentration of 5,300 mg/L and 5,500 mg/L was observed with 8.3 % variation. The HPC were observed to be close between each observed MLSS, which could be due to narrow range in the concentration levels maintained. Although, it was generally observed that HPC for each particular MLSS concentration increased with increasing MLSS concentration, which possibly could indicate viable cells quantity for the microorganisms in the aeration chamber intensifying with MLSS concentration level due to possible increase in organic loading and metabolic activities.

4.6.3.2 Phase III results for protozoa and metazoa counts in aeration chamber sludge

Random samples were grabbed in the aeration chamber for microbial examination in phase III. The samples were analysed during each sub phase of steady state operation. Phase III was operated with average AER-C MLSS concentration observed to be 5,139 mg/L, F/M ratio during the operation was observed to be in the range of 0.49-0.79 kg COD/kg MLVSS d, and AER-C OLR was observed to be in range of 0.06-0.09 kg COD/m^3. d. The experiment was conducted in triplicates of 100 µL sub samples of the sludge samples analysed. The results are presented as average and percentage for the population of microfauna existing in the sludge.

Nematodes were observed to exist from phase III-A through phase III-E after operating bioreactor for long SRT of 40 days. Count of micro-organisms observed in phase III-A through phase III-E is presented in the Table 4.22. Metazoa population observed consist of nematodes and rotifers constituting between 18.8-25.3 % (180-260 organisms/mL). Nematodes population observed was between 1.0-2.1 % (10-100 organisms/mL), and population of rotifers was observed to be between 17.3-23.9 % (170-330 org/mL). Water bears were observed during phase III-D and phase III-E of the operation with low percentage population of 1 % (average of 10 org/mL of sample). Water bears could be found in the same environment with rotifers and nematodes. Presence of water bear usually indicates that there is little to no ammonia present [333]. This could be a good indication that system did not inhibit nitrification, which is probably suggested by the long operational SRT, and other

favorable factors for system such as controlled pH. However, microorganisms such as free swimmers and stalk ciliates were observed to dominate the ASP. Population of free swimming ciliate and stalked ciliates were observed within the ranges of 29.7-37.8 % (190-370 org/mL) and 41.2-48.4 % (310-490 org/mL), respectively.

Table 4.22: Protozoa and Metazoa count in aeration chamber sludge

Organism	Slide no. 1	Slide No. 2	Slide no. 3	Aver.	Percentage (%)	
Protozoa						
Free-swimming ciliates	16	20	13	16	33.3	79.1
Stalked ciliates	22	18	27	22	45.8	
Metazoa						
Rotifers	9	7	12	9	18.8	20.9
Nematodes	2	1	1	1	2.1	
Total	49	46	53	48	100	100
Phase III-B						
Protozoa						
Free-swimming ciliates	23	14	19	19	29.7	78.1
Stalked ciliates	31	38	25	31	48.4	
Metazoa						
Rotifers	12	9	17	13	20.3	21.9
Nematodes	1	2	1	1	1.6	
Total	67	63	62	64	100	100
Phase III-C						
Protozoa						
Free-swimming ciliates	38	33	40	37	37.8	81.7
Stalked ciliates	46	40	44	43	43.9	
Metazoa						
Rotifers	17	21	14	17	17.3	18.3
Nematodes	1	1	1	1	1.0	
Total	102	95	99	98	100	100

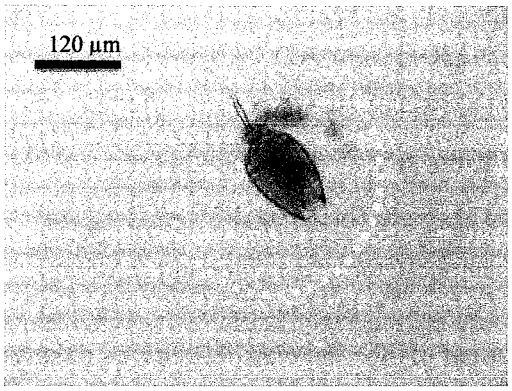
Table 4.22: Protozoa and Metazoa count in aeration chamber sludge cont.

Phase III-D						
Protozoa						
Free-swimming ciliates	44	40	37	40	33.6	74.8
Stalked ciliates	48	54	45	49	41.2	
Metazoa						
Rotifers	25	32	28	28	23.5	25.1
Nematodes	2	1	1	1	0.8	
Water bear	1	1	1	1	0.8	
Total	120	128	112	119	100	100
Phase III-E						
Protozoa						
Free-swimming ciliates	48	39	44	44	31.9	74.7
Stalked ciliates	65	53	60	59	42.8	
Metazoa						
Rotifers	29	32	37	33	23.9	25.3
Nematodes	1	2	1	1	0.7	
Water bear	1	1	NI	1	0.7	
Total	144	127	142	138	100	100
NI = Not identifiable						

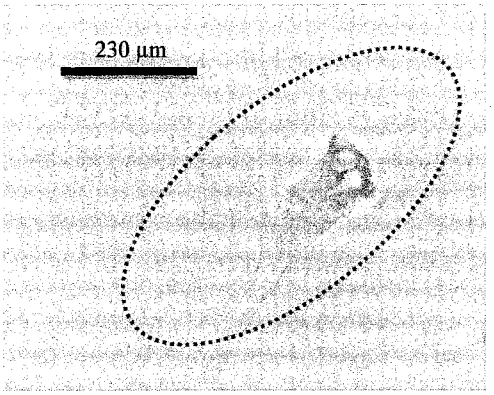
Population of stalked ciliates dominated from the entire population observed. Papadimitriou et al. reported a shift of dominant protozoan populations was observed from free-swimming species during early operation towards stalked ciliates in extended periods of the operation [334]. This change in protozoan composition with the time of operation has also been mentioned in sewage treatment plants [335]. When most of nutrients present in wastewater are reduced ciliates will dominate, and stalked ciliates and metazoa will be predominant in longer SRT systems mainly because of their ability to compete, when very little nutrients are left and their capacity to feed on other protozoa [334].

Some of significant protozoa and metazoa observed from phase III-A through phase III-E are presented in Figure 4.53. The organisms were viewed under 400x magnification optical microscope. They consist of free swimming ciliates, stalked ciliates, rotifers, nemotodes and water bear. The organism indicated in Figure 4.53a is Free swimming ciliate, stalked ciliate are shown if Figure 4.53b, Rotifiers are shown in

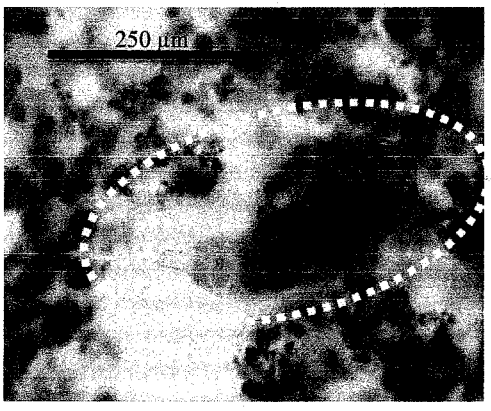
Figure 4.53c, nematodes are shown in Figure 4.53d, water bear is shown in Figure 4.53e, and with side view of water bear shown in Figure 4.53f.



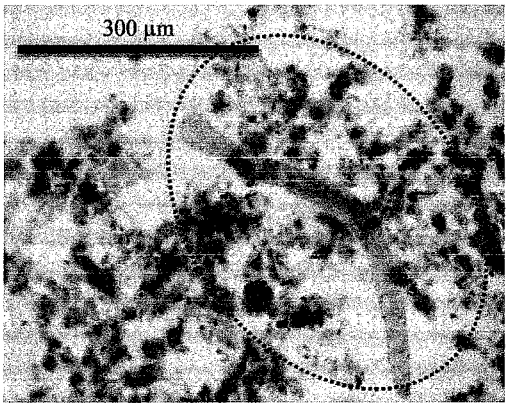
a. Free swimming ciliate (x400)



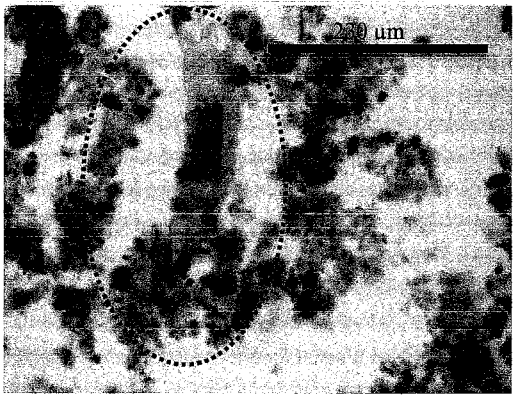
b. Stalked ciliate (x400)



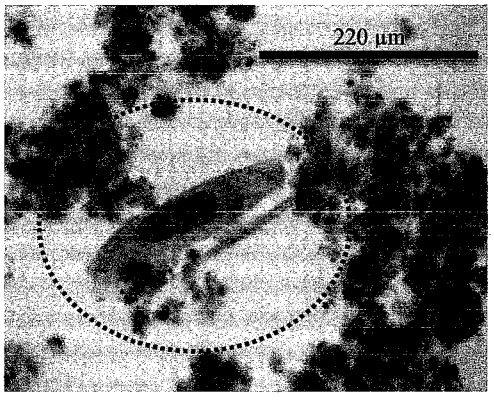
c. Rotifer (x400)



d. Nemotode (x400)



e. Water bear (x400)



f. Water bear side view (x400)

Figure 4.53: a, b, c, d, e and f illustrate some protozoa and metazoan observed

4.6.4 Phase III results for pH, dissolved oxygen and temperature profiles

The pH, DO, and temperature values were monitored and measured during phase III operation. pH was measured in the influent, ANX-C, AER-C and AD-C. DO was monitored and measured in the ANX-C, AER-C, and AD-C. The temperature was measured in situ from the AER-C of the i-SGBR system. Data for pH, DO and temperature can be found in Appendix B (Table B 1.2). To ensure pH control close to neutral was achieved, doses of sodium bicarbonate ($NaHCO_3$) in AER-C and AD-C were frequently added. The experiment was conducted at an ambient temperature.

In phase III-A through phase III-E, influent pH for the wastewater was observed to be in the range between 4.4 to 6.6, with an average value and standard deviation of 5.9±0.6. The profile for the influent pH in phase III is as shown in Figure 4.54.

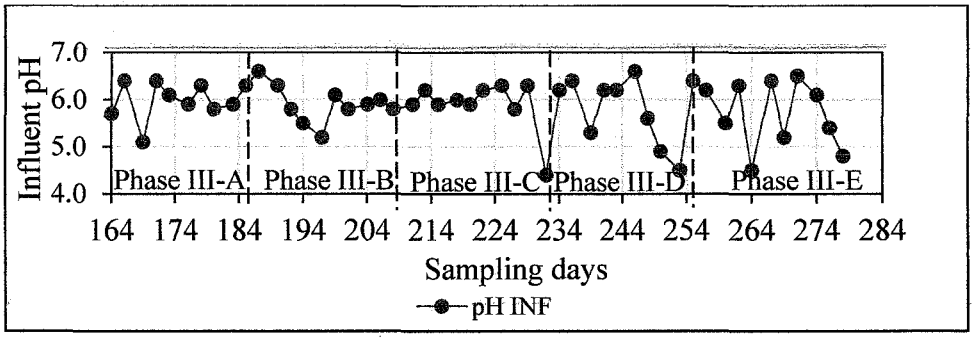


Figure 4.54: pH profile for influent wastewater vs Sampling Days

pH for ANX-C in phase III-A through phase III-E was observed to be in the range between 7.2-7.7, with observed average value and standard deviation obtained as 7.5±0.1. pH profile for ANX-C in phase III is shown in Figure 4.55.

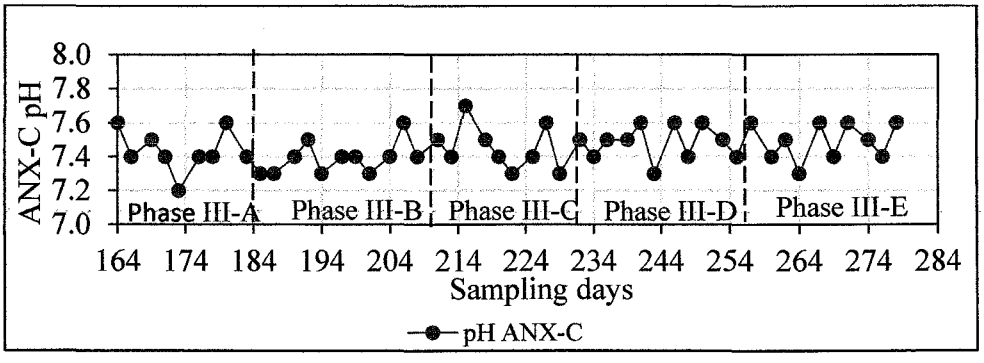


Figure 4.55: pH profile for Anoxic chamber vs Sampling Days

The observed pH for AER-C in phase III-A through phase III-E ranges between 7.2-7.6, with an average value and standard deviation obtained as 7.2±0.1. pH profile for the AER-C is as shown in Figure 4.56.

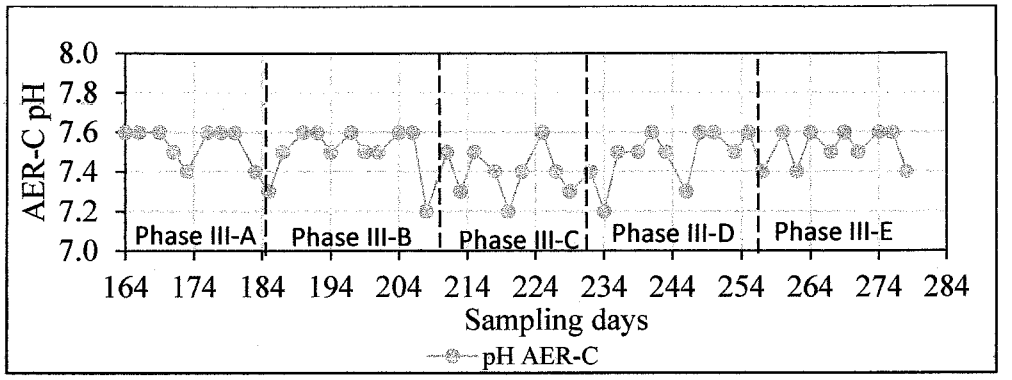


Figure 4.56: pH profile for Aeration chamber vs Sampling Days

It can be observed from Figure 4.55 and Figure 4.56 that pH close to neutral was maintained in reaction chambers throughout operation in phase III. Influent pH of the wastewater was observed to be slightly acidic as can be seen from Figure 4.54. This might be due to the nature of the wastewater by-products and cleaning operations from the FBI wastewater. However, pH in the i-SGBR system was maintained within a stable range of 6.8-7.8, which could be favorable for microbial activities. Tolerable pH for microbial process ranges between 6.0 to 9.0, and optimum performance occurs close to neutral pH [89, 90].

DO in the ANX-C for phase III-A through phase III-E ranges between 0.21 mg/L to 0.28 mg/L, with average and standard deviation recorded as 0.24±0.02 mg/L. DO profile for phase III-A through phase III-E in the ANX-C is shown in Figure 4.57.

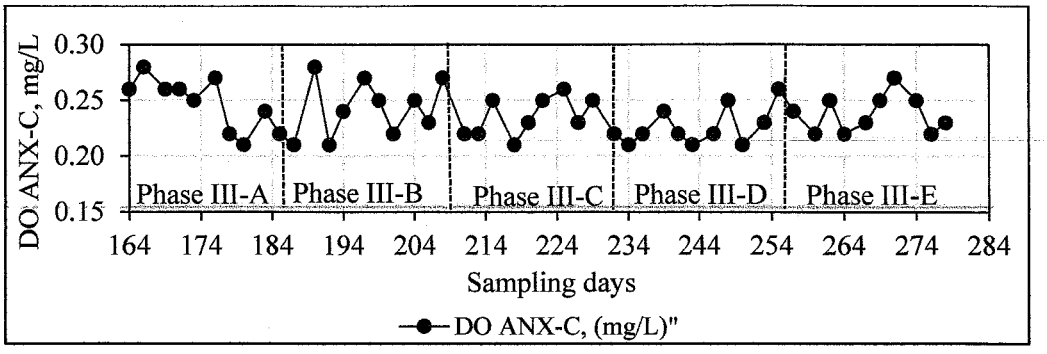


Figure 4.57: Anoxic chamber dissolve oxygen conc. vs Sampling Days

DO for AER-C in phase III-A through phase III-E ranges between 3.98 mg/L and 4.98 mg/L with an average and standard deviation of 3.98±0.3 mg/L. DO profile for AER-C for phase III-A through phase III-E is shown in Figure 4.58. Fine bubble diffusers in AER-C served dual purpose, providing sufficient mixing and supply of DO.

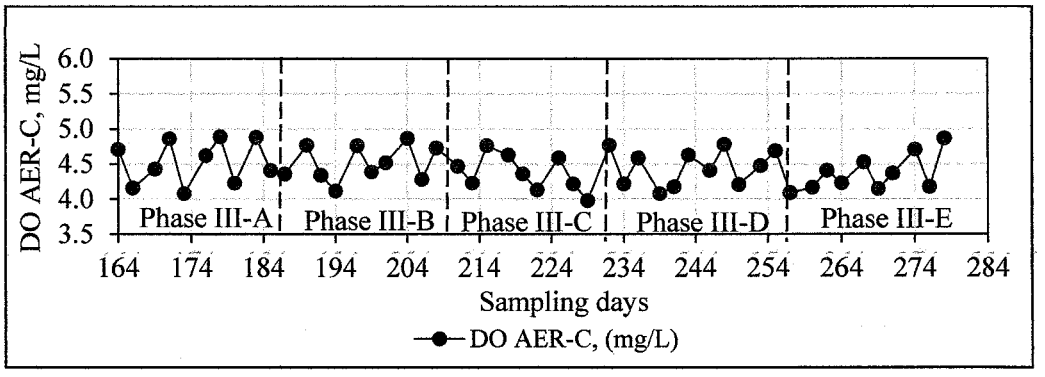


Figure 4.58: Aeration chamber dissolve oxygen conc. vs Sampling Days

The DO was strictly maintained in AER-C and fluctuations throughout phase III was insignificant as can be seen from Figure 4.58. However, the slight variations of DO observed might be attributable to requirements for oxidation of organic matter and nitrification in the aerobic chamber, due to variable wastewater influent organic loadings. DO concentration was always adjusted by gauge valves to ensure required and controlled supply.

Temperature was monitored from the AER-C of i-SGBR system in phase III-A through phase III-E, where the observed values range between 27.4 and 33.3 °C. The average and standard deviation were recorded as 29.9±1.1 °C. Biochemical reactions

could be influenced by temperature. Temperature profile vs sampling days in AER-C is shown in Figure 4.59.

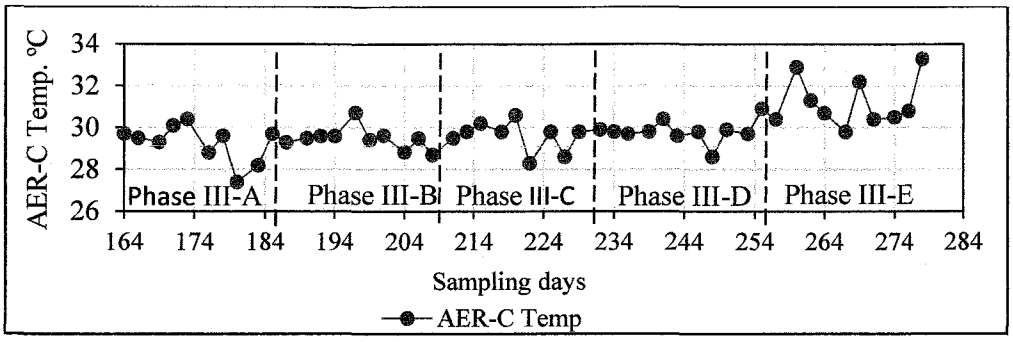


Figure 4.59: Aeration chamber temperature vs Sampling Days

4.7 Phase III results on performance for system operation

In this research, data for the performance analysis considers the steady state periods at respective stages in phase III. Phase III was conducted between day 162 to 278. Five flow rates performance was evaluated for the removal of organic matter (COD, sCOD, BOD_5) and nitrogen (ammonia-nitrogen, nitrate-nitrogen, and nitrite). Samples were obtained from the influent (INF), effluent anoxic chamber (E-ANX), and effluent aeration chamber (E-AER), return activated sludge (RAS), and an effluent clarifier (E-CLR). Steady state data was obtained considering values for standard deviation with less than 10 % removal efficiencies of COD, nitrification, and denitrification as highlighted in Section 3.4. The detailed performance data for the duration of the experiment including parameters for COD, sCOD, BOD_5, TSS, ammonia-nitrogen, nitrate, nitrite, TKN and TN are contained in Appendix D (Table D1.1 through Table D1.6).

Operation in phase III was conducted with five variable flow rates and SRT. These flow rates range between 100 to 150 L/d, and the SRT range 20 to 40 days. The five variable flow rates produced corresponding OLR rates and HRT for the ANX-C and AER-C. The corresponding ANX-C and AER-C HRT for the operation ranges between 6.4 to 9.6 hrs, and between 20 to 30 days HRT for the extended aeration, respectively. HRT for AD-C was maintained at constant rate irrespective of OLR and bioreactor HRT operated. Internal recycle ratio of 6 and RAS ratio of 0.5 were maintained throughout

phase III. IR flow ranges between 600 L/d in phase III-A to 900 L/d in phase III-E. Average AER-C MLSS and MLVSS were in the range of 5010 to 5237 mg/L, and 3021 to 3227 mg/L, respectively. This gives AER-C MLSS/MLVSS ratio within the range of 0.60 and 0.61. The underflow sludge concentration with MVLSS and MLVSS concentrations were observed to be within the range of 13,055 to 19,475 mg/L, and 10,893 to 15,021 mg/L, respectively. Correspondingly, this gives average MLVSS to MLSS ratio as 0.77 to 0.85, respectively. The performance was evaluated based on operational variables with specific details of each stage as shown in Table 4.23.

Table 4.23: phase III results for operational conditions of biokinetic study

Phase	III				
Experimental stage	III-A	III-B	III-C	III-D	III-E
Steady state period (days)	173-185	199-208	208-232	222-255	246-278
Flow rate, Q_{INF}, (L/d)	100	110	120	135	150
Hydraulic retention time Θ_{ANX-C}, HRT (hours)	9.6	8.7	8.0	7.1	6.4
Hydraulic retention time Θ_{AER-C}, HRT (hours)	30	27.3	25.0	22.2	20.0
Hydraulic retention time Θ_{I-SGBR}, HRT (days)	2.65	2.41	2.21	1.96	1.77
Hydraulic retention time, Clarifier, Θ_{CLAR} (days)	1.00	0.91	0.83	0.74	0.67
Solids retention time Θ_c, AER-C of SRT_{I-SGBR} (days)	40.8±0.7	35.9±0.5	30.5±0.8	25.2±0.7	20.0±0.3
AD-C SRT, (days)	10				
Internal recycle flow= Q_{INF} *IR, (L/d)	600	660	720	810	900
MLSS in AER-C, mg/L	5010±65	5070±89	5166±151	5212±119	5237±92
MLVSS in AER-C, mg/L	3021±88	3085±124	3104±142	3178±176	3227±112
MLSS in RAS, mg/L	13,055±360	13,917±294	15,622±270	18,107±169	19,475±236
MLVSS in RAS, mg/L	10,893±257	11,609±275	13,113±326	14,606±113	15,021±197
MLVSS/MLSS ratio AER-C	0.60	0.60	0.60	0.60	0.61
MLVSS/MLSS ratio RAS	0.85	0.83	0.84	0.81	0.77
Total RAS flow (R_{Total}) = (R_i) * Q_{INF}, (L/d)	50	55	60	70	75

4.7.1 Phase III results for removal of COD, sCOD, and BOD

In order to monitor the degradation of organic matter, evaluation for the removal of organic matter was carried out based on the COD, sCOD, and BOD_5. Figure 4.60 through Figure 4.62 shows the result of the influent COD and effluent COD, ANX-C and AER-C OLR, and COD removal efficiency from phase III-A through phase III-E, respectively.

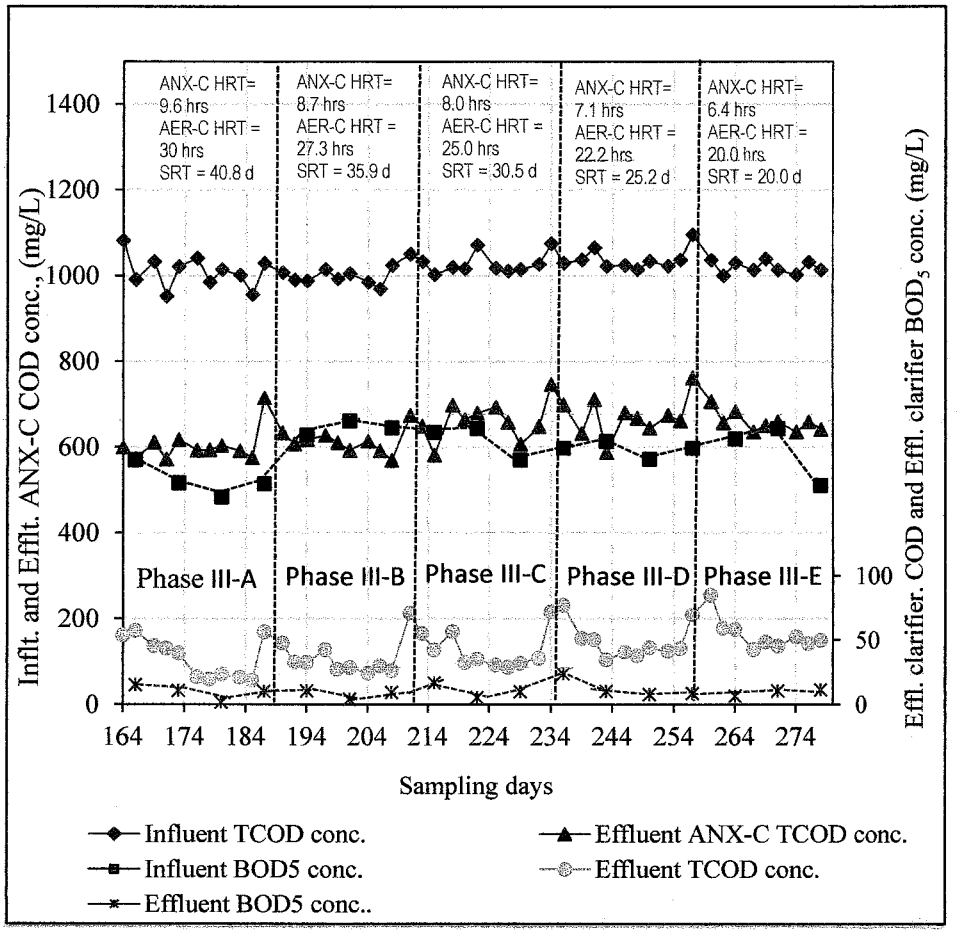


Figure 4.60: Infl., Effl.-Anoxic & Effl.-Clarifier COD and Infl. & Effl.-Clarifier. BOD_5 conc. vs Sampling Days

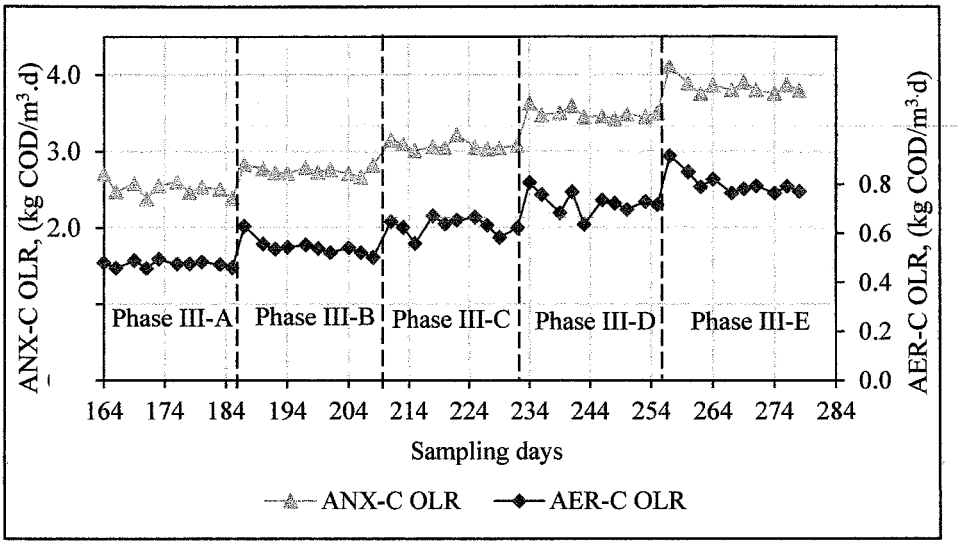


Figure 4.61: Anoxic and Aeration organic loading rate vs Sampling Days

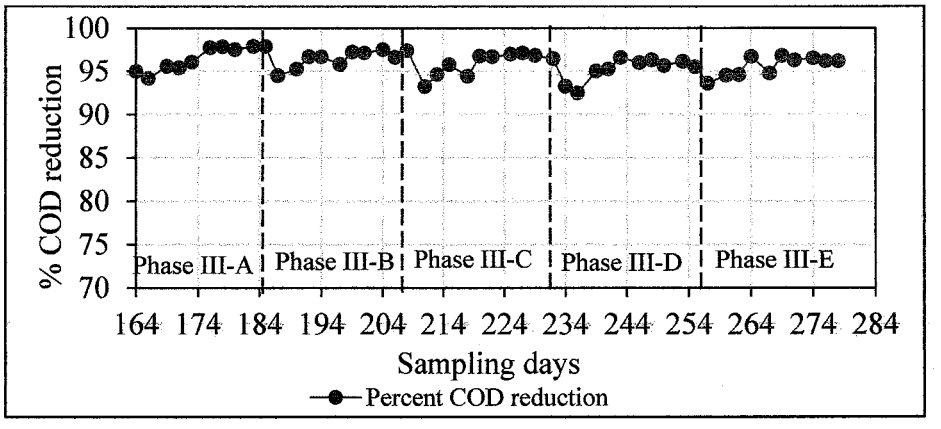


Figure 4.62: COD removal percentage vs. Sampling days

In phase III-A operation, steady state was observed between days 173 to day 185 in effluent-ANX-C (E-ANX-C), effluent-AER-C (E-AER-C), and effluent clarifier (E-CLR). At observed steady state, average and standard deviation values for COD concentrations in E-ANX-C and E-CLR were observed to be 591±10 and 21±2 mg/L, respectively. This gives 97.9 % system removal efficiency with equivalent observed influent COD concentration average and standard deviation of 1003±30 mg/L. Corresponding BOD_5 concentration for influent and E-CLR were observed with average and standard deviation of 500±23 and 6.3±7 mg/L, respectively. The untreated influent and treated E-CLR BOD_5/COD ratio at steady state period gives 0.5 and 0.21, respectively. The opretional ANX-C and AER-C OLR observed were 2.52±0.1 and 0.49±0.01 mg COD/m^3 d, respectively. The F/M ratio for the AER-C was observed as

0.06 mg sCOD/mg MLVSS d based on sCOD concentration. It was observed that about 41 % of COD was reduced due to biological degradation in ANX-C. Specific COD removal rate in ANX-C of 0.72±0.03 mg COD/mg VSS d was observed. As a result, because COD was probably used in ANX-C for denitrification, balance was degraded aerobically in AER-C. Average specific COD removal rate in AER-C at steady state was determined to be 0.41±0.03 mg COD/mg VSS d.

In phase III-B operation, ANX-C and AER-C OLR were operated with observed average and standard deviation of 2.75±0.1 and 0.54±0.03 kg COD/m^3.d, respectively. Between day 199 to day 208, steady state was observed, where E-ANX-C and E-CLR had average and standard deviation COD concentration of 596±18 and 28±3 mg/L, respectively. It was observed that 40 % of COD was degraded in ANX-C. Equivalent BOD_5 concentration in E-CLR was observed with average value and standard deviation of 6.8±4 mg/L. Influent concentration for COD and BOD_5 had average values and standard deviation observed to be 995±21 and 653±11 mg/L, respectively. This gives untreated and treated BOD_5/COD ratios of 0.7 and 0.4, respectively. It can be realized that system has achieved 97.2 % COD removal efficiency. Average value and standard deviation of specific COD removal rate in ANX-C and AER-C were observed to be 0.39±0.04 and 0.115±0.001 mg COD/mg VSS d, respectively. Low BOD_5 values observed could deduce that wastewater contains high biodegradable organic content.

In phase III-C, steady state was observed with E-CLR average and standard deviation COD of 33±3 mg/L. Observed influent COD concentration average and standard deviation were 1028±25 mg/L, which represents 96.8 % system COD removal efficiency. ANX-C COD utilization was observed with average of 36 %, E-ANX-C COD concentration was observed to be 657±33 mg/L. The equivalent average and standard deviation for influent and E-CLR BOD_5 concentrations were observed as 607±62 and 7.8±3 mg/L, respectively. This gives untreated and treated BOD_5/COD ratios of 0.6 and 0.24, respectively. ANX-C and AER-C COD OLR were operated with average value and standard deviation of 2.75±0.05 and 0.54±0.03 mg COD/m^3 d, respectively. F/M ratio in AER-C was observed to be 0.08 mg sCOD/mg MLVSS d. Average and standard deviation of specific COD removal rate in ANX-C and AER-C were determined to be 0.29±0.03 and 0.12±0.01 mg COD/mg VSS d, respectively.

In phase III-D operation, steady state was observed between day 222 to day 232 with operational ANX-C and AER-C OLR of 3.08±0.1 and 0.62±0.03 mg COD/m^3 d, respectively. E-CLR COD concentration had average and standard deviation observed as 42±4 mg/L. Observed influent and E-ANX-C COD concentration had average and standard deviation of 1026±9 and 666±14 mg/L, respectively. System COD removal efficiency of 95.9 % was observed. ANX-C COD utilization was observed at average 35 %. The equivalent average and standard deviation for influent and E-CLR BOD_5 were observed to be 585±18 and 8.3±0.7 mg/L, respectively. This gives untreated and treated BOD_5/COD ratios of 0.6 and 0.2, respectively. ANX-C and AER-C COD OLR were observed with an average and standard deviation of 3.50±0.07 and 0.71±0.05 mg COD/m^3d, respectively. F/M ratio in AER-C was observed to be 0.09 mg sCOD/mg MLVSS d. The average value and standard deviation for specific COD removal rate in ANX-C and AER-C were observed as 0.27±0.02 and 0.13±0.01 mg COD/mg VSS d, respectively.

In phase III-E, steady state was observed between day 269 to day 278 with E-CLR COD concentration average and standard deviation of 49±3 mg/L. The observed influent COD concentration average and standard deviation was 1020±15 mg/L, which represents 95.2 % system COD removal efficiency. ANX-C COD utilization was observed at an average of 36.3 %, where E-ANX-C COD concentration was observed to be 650±11 mg/L. The equivalent average and standard deviation for influent and E-CLR BOD_5 were observed to be 577±94 and 10.2±0.8 mg/L, respectively. This gives untreated and treated BOD_5/COD ratios of 0.6 and 0.3, respectively. The ANX-C and AER-C COD OLR were observed with an average value and standard deviation of 3.85±0.1 and 0.79±0.04 mg COD/m^3d, respectively. F/M ratio in AER-C was observed to be 0.09 mg sCOD/mg MLVSS d. Average and standard deviation for specific COD removal rate in ANX-C and AER-C were obtained as 0.27±0.01 and 0.14±0.004 mg COD/mg VSS d, respectively.

A statistical analysis (ANOVA) was conducted on the E-CLR average COD concentration values obtained from the experimental data. At 95% confidence level, the result indicated significant difference ($P<0.05$) between the mean values of the concentration at respective HRT's (Table 4.24), where the results have shown that mean

value for HRT with flow rate of 100 L/d was lower than the mean values of other flow rates having 110, 120, 135 and 150 L/d. Hence, HRT with flow rate of 100 L/d achieved better performance of COD removal compared to other HRT's with flow rates of 110, 120, 135 and 150 L/d.

Table 4.24: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on COD performance

Command Window

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```
>> [p,tbl,stats] = anova1(COD2)

p =

  4.9716e-013


tbl =

  Columns 1 through 5

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                []


stats =

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         n: [5 5 5 5 5]
    source: 'anova1'
     means: [20.8000 27.3200 32.6000 41.4600 48.8000]
        df: 20
         s: 2.4572
```

Table 4.24: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on COD performance cont.

```
>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000  -11.1704   -6.5200   -1.8696
    1.0000    3.0000  -16.4504  -11.8000   -7.1496
    1.0000    4.0000  -25.3104  -20.6600  -16.0096
    1.0000    5.0000  -32.6504  -28.0000  -23.3496
    2.0000    3.0000   -9.9304   -5.2800   -0.6296
    2.0000    4.0000  -18.7904  -14.1400   -9.4896
    2.0000    5.0000  -26.1304  -21.4800  -16.8296
    3.0000    4.0000  -13.5104   -8.8600   -4.2096
    3.0000    5.0000  -20.8504  -16.2000  -11.5496
    4.0000    5.0000  -11.9904   -7.3400   -2.6896

m =

   20.8000    1.0989
   27.3200    1.0989
   32.6000    1.0989
   41.4600    1.0989
   48.8000    1.0989

fx >>
```

4.7.1.1 Phase III results for sCOD concentration reduction profile

The soluble COD (sCOD) for the whole of phase III study from day 164 to 278 is presented in Figure 4.63. Samples were obtained from influent, E-ANX-C, E-AER-C and E-CLR. The influent sCOD concentration for the period was observed with minimum, maximum and average values of 385, 467 and 425 mg/L, respectively.

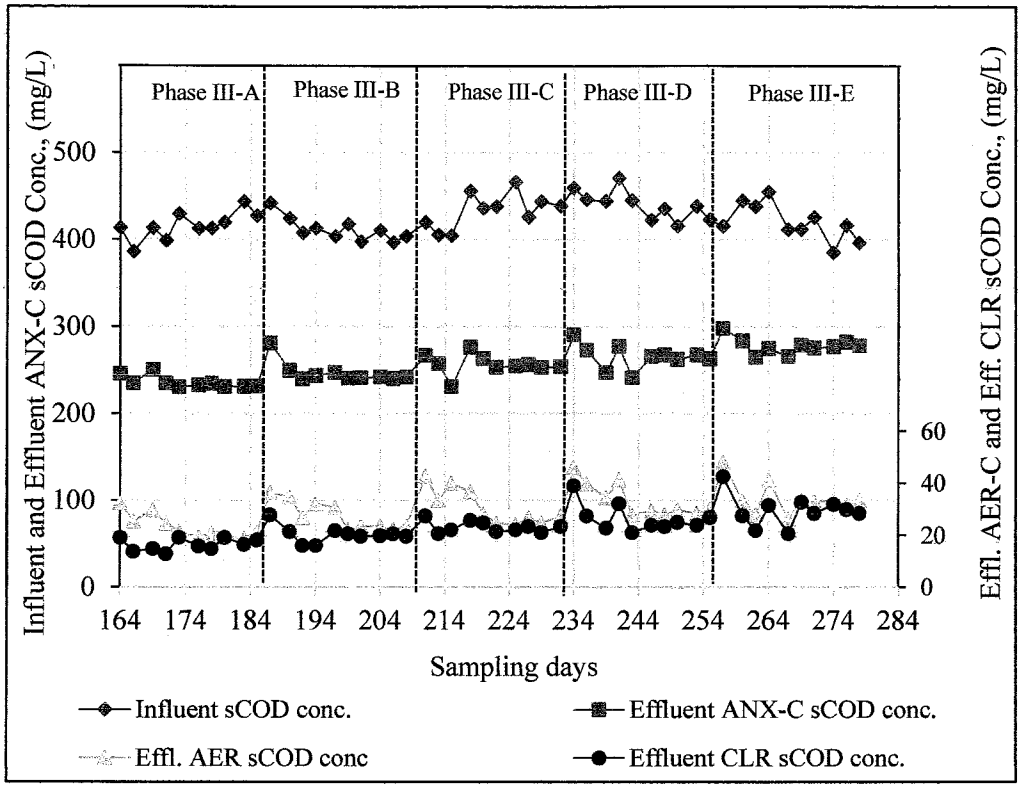

Figure 4.63: sCOD conc: Inf., E-ANX-C, E-AER-C, and E- CLR. vs Samp. Days

In phase III-A operation, steady state was observed between day 173 to 185. Measured concentration in the influent, E-ANX-C, E-AER-C and effluent clarifier, where observed with average values and standard deviation of 424±12, 232±1.7, 20±2, and 17±1 mg/L, respectively. It can be seen that sCOD removal efficiency of 92.6 % sCOD was achieved. The influent and effluent COD/sCOD ratio was observed to be 2.3 and 1.3, respectively.

Between day 199 to 208, steady state was observed for phase III-B operation. The average influent concentration of sCOD was observed as 406±9 mg/L. This gives COD/sCOD ratio of 2.4. Average concentration and standard deviation in E-ANX-C, E-AER-C and E-CLR were observed as 241±7, 22.7±1.4 and 20±2 mg/L, respectively. Removal efficiency of 91.7 % sCOD was observed for the system. Effluent COD/sCOD ratio was observed to be 1.7.

In phase III-C operation, steady state was observed between day 222 to day 232. The average influent sCOD for the operation was observed with an average value and

standard deviation of 443±15 mg/L, with corresponding E-ANX-C, E-AER-C and E-CLR observed as 254±13, 25.5±2 and 24±1 mg/L, respectively. This gives influent and effluent COD/sCOD ratio as 2.3 and 1.7, respectively. sCOD removal efficiency of 90.6 % was observed.

In phase III-D operation, steady state was observed between day 246 to 255. The concentration in influent, E-ANX-C, E-AER-C and E-CLR, where observed with average values and standard deviation of 427±10, 265±8, 28±2, and 25±3 mg/L, respectively. sCOD removal efficiency of 90.6 % sCOD was observed for the system. The influent and effluent COD/sCOD ratio was observed to be 2.4 and 1.8, respectively.

Between day 269 to 278, steady state was observed for phase III-E operation. The average influent concentration of sCOD was observed as 407±16 mg/L. This gives COD/sCOD ratio of 2.5. The corresponding average concentration and standard deviation for E- ANX-C, E-AER-C and effluent clarifier were observed as 278±2, 33.1±0.8 and 29±2 mg/L, respectively. The removal efficiency of 89.5 % sCOD was observed for the system. The influent and effluent COD/sCOD ratio was observed to be 2.5 and 1.8, respectively.

A statistical analysis (ANOVA) was conducted on both E-ANX-C sCOD and E-AER-C sCOD based on average concentration values obtained from the experimental data. At 95% confidence level, the result has indicated significant difference ($P<0.05$) between the mean values of the concentration at respective HRT's (Table 4.25 and Table 4.26), where the results indicated that the mean value for HRT with flow rate of 100 L/d was lower than the mean values of other flow rates having 110, 120, 135 and 150 L/d. Hence, HRT with flow rate of 100 L/d achieved better performance of sCOD removal compared to HRT with flow rates of 110, 120 , 135 and 150 L/d.

Table 4.25: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on sCOD (E-ANX-C) performance

```
New to MATLAB? Watch this video, see Demos, or read Getting Started.

>> [p,tbl,stats] = anoval (SCODEANOX)

p =

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tbl =

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  Column 6

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          []
          []


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           n: [5 5 5 5 5]
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           s: 1.8921

>> [c,m] = multcompare(stats)

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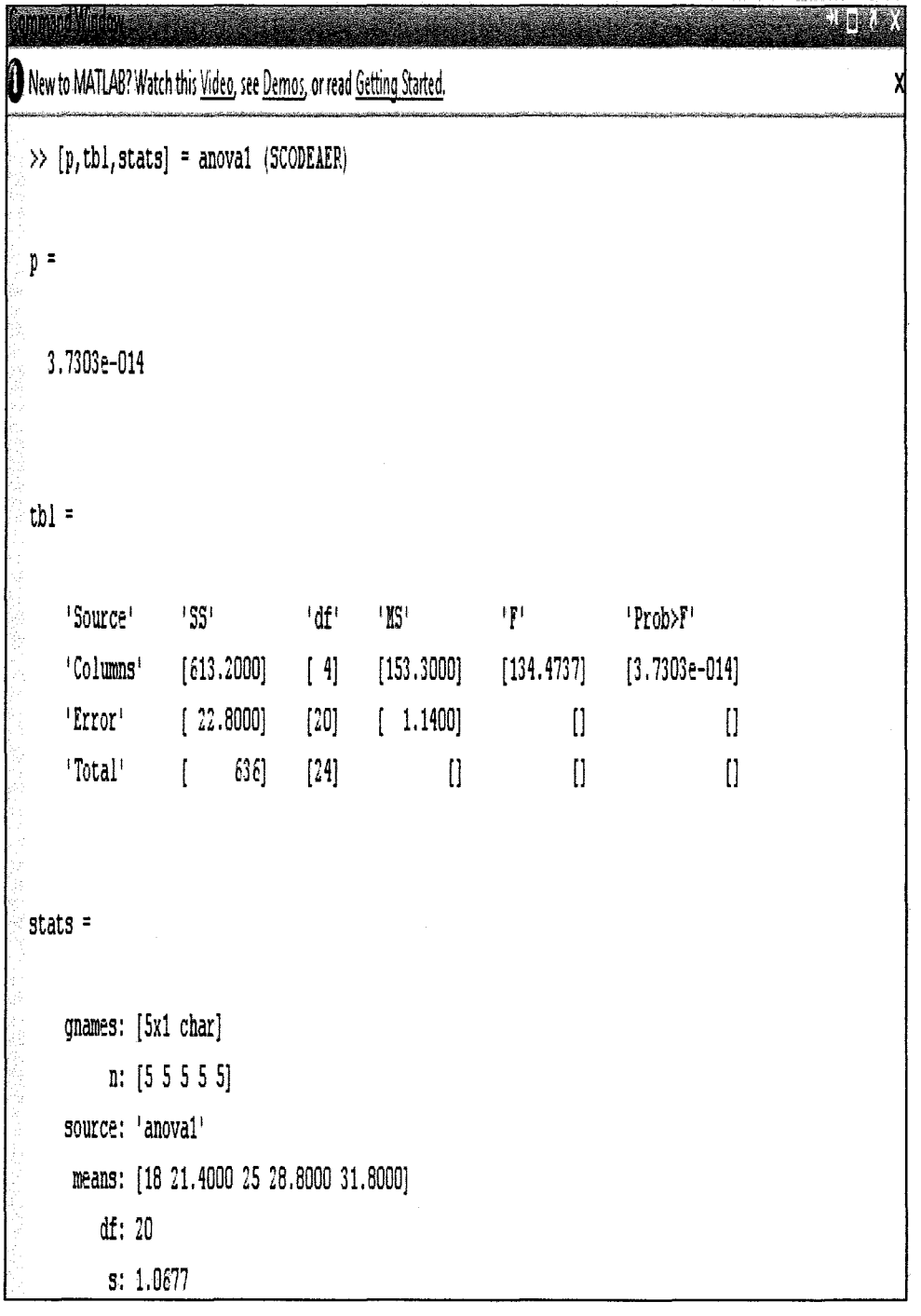
    1.0000    5.0000   -40.5809   -34.0000   -27.4191
    2.0000    3.0000   -16.7809   -15.2000   -9.6191
    2.0000    4.0000   -27.9809   -24.4000   -20.8191
    2.0000    5.0000   -40.7809   -37.2000   -33.6191
    3.0000    4.0000   -14.7809   -11.5000   -7.4191
    3.0000    5.0000   -27.5809   -24.0000   -20.4191
    4.0000    5.0000   -16.3809   -12.8000   -9.2191


m =

  232.4000    0.8462
  241.0000    0.8462
  254.4000    0.8462
  265.6000    0.8462
  278.4000    0.8462
```

Table 4.26: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on sCOD (E-AER-C) performance.



```
Command Window
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>> [p,tbl,stats] = anova1 (SCODEAER)

p =

  3.7303e-014


tbl =

    'Source'    'SS'          'df'    'MS'          'F'          'Prob>F'
    'Columns'   [613.2000]    [ 4]    [153.3000]    [134.4737]   [3.7303e-014]
    'Error'     [ 22.8000]    [20]    [  1.1400]          []             []
    'Total'     [      636]   [24]            []          []             []


stats =

    gnames: [5x1 char]
         n: [5 5 5 5 5]
    source: 'anova1'
     means: [18 21.4000 25 28.8000 31.8000]
        df: 20
         s: 1.0677
```

Table 4.26: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on sCOD (E-AER-C) performance cont.

```
>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -5.4207   -3.4000   -1.3793
    1.0000    3.0000   -9.0207   -7.0000   -4.9793
    1.0000    4.0000  -12.8207  -10.8000   -8.7793
    1.0000    5.0000  -15.8207  -13.8000  -11.7793
    2.0000    3.0000   -5.6207   -3.6000   -1.5793
    2.0000    4.0000   -9.4207   -7.4000   -5.3793
    2.0000    5.0000  -12.4207  -10.4000   -8.3793
    3.0000    4.0000   -5.8207   -3.8000   -1.7793
    3.0000    5.0000   -8.8207   -6.8000   -4.7793
    4.0000    5.0000   -5.0207   -3.0000   -0.9793


m =

   18.0000    0.4775
   21.4000    0.4775
   25.0000    0.4775
   28.8000    0.4775
   31.8000    0.4775
```

4.7.2 Phase III results for removal of Total suspended solids

Phase III experiment to measure both influent and effluent TSS was carried out between days 164 to 278. TSS was monitored from influent and effluent to enumerate the quality for course of sedimentation process in the clarifier. Clarifier functioned primarily to settle the sludge resulting from the secondary treatment. Influent and effluent TSS results for the study period in phase III is shown in Figure 4.64. Five TSS loadings were applied corresponding to five system HRT's of 2.65 days, 2.41 days, 2.21 days, 1.96 days and 1.77 days corresponding to phase III-A, phase III-B, phase III-C, phase III-D and phase III-E, respectively.

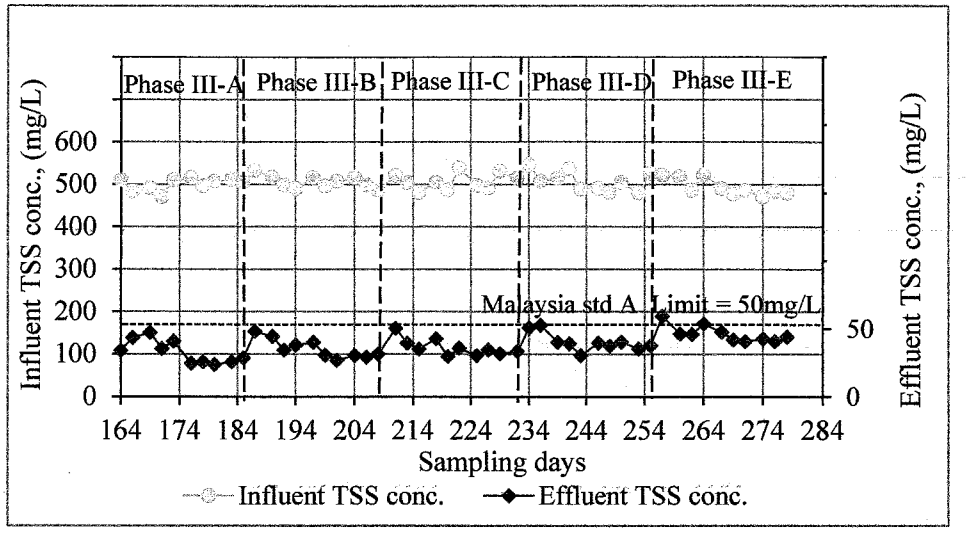

Figure 4.64: Influent and effluent TSS vs Sampling Days

The concentration of influent TSS (I-TSS) for whole of phase III ranges between 489±19 mg/L, which was observed in phase III-D to 507±7 mg/L observed in phase III-A. The average and standard deviation of TSS concentration over the time course of phase III experiment were observed as 504±20 mg/L. The interpretation of TSS results in phase III according to Figure 4.64 is detailed in Figure 4.65 with respective steady state average values. The error bar represents standard deviation.

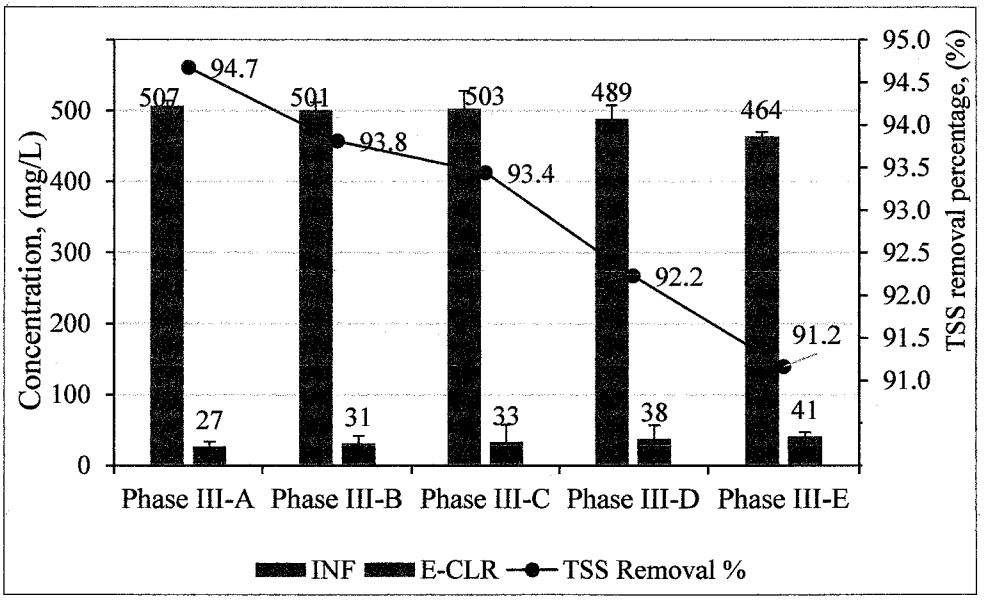

Figure 4.65: Interpretation of results for Infl. and Effl. TSS conc.

From day 173 to 183 when steady state was observed in phase III-A, effluent TSS (E-TSS) concentration was observed with average value and standard deviation of 27±2 mg/L. Considering I-TSS concentration with average value and standard deviation of 507±7 mg/L, TSS reduction of 94.7 % was achieved with system HRT of 2.65 days. This gives COD/TSS ratio of 1.98 and 0.77 in influent and effluent, respectively.

From day 199 to 208 in phase III-B when steady state was observed, E-TSS with an average value and standard deviation of 31±3 mg/L was observed. Corresponding I-TSS average value and standard deviation was 501±11 mg/L. TSS removal efficiency of 93.8 % was observed with system HRT of 2.41 days. Corresponding COD/TSS ratio in influent and effluent was observed to be 1.98 and 0.90, respectively.

From day 222 to day 232 when phase III-C operation was carried out, E-TSS average concentration was observed to be 33±2 mg/L with equivalent I-TSS concentration observed with an average value of 503±25 mg/L. This gives removal efficiency of 93.4 %. TSS removal from phase III-B and phase III-C though closely different, but increased stratification of sludge and reduced biomass washout could improve the E-TSS quality. The ratio for the influent and effluent COD/TSS was observed as 2.04 and 1.0, respectively.

From day 246 to 255 when steady state operation was observed in phase III-D, effluent clarifier TSS concentration of 38±2 mg/L was observed, with a corresponding I-TSS concentration of 489±19 mg/L. Similarly, TSS removal efficiency of 92.2 mg/L was observed. This gives COD TSS for influent and effluent as 2.10 and 1.11, respectively.

Between day 269 to 278 steady state was observed in phase III-E, with average E-TSS concentration of 41±1.8 mg/L. The corresponding I-TSS concentration was observed with an average value and standard deviation of 464±6 mg/L. TSS removal efficiency of 91.1 % was observed.

Enhanced TSS removal could be attributed to the combination of complete retention of particulate organic matter (COD or BOD_5) in the clarifier, including the suspended

organic matter and high molecular weight organisms, thus prevention of sludge washout problems, which is normally common to activated sludge systems.

A statistical analysis (ANOVA) was conducted on E-TSS results obtained from the experimental data. At 95% confidence level, the result has shown that significant difference ($P<0.05$) between the mean values of the TSS exist at respective HRT's operated (Table 4.27), where the results indicated that the mean value for HRT with flow rate of 100 L/d was lower than the mean values of other flow rates having 110, 120, 135 and 150 L/d.. It can be concluded that HRT with a flow rate of 100 L/d achieved better performance of TSS removal compared to HRT with flow rates of 110, 120, 135 and 150 L/d.. Consequently, gradual decline in performance of TSS removal efficiency was observed with gradual increase in OLR and decrease in HRT.

Table 4.27: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on TSS performance

Command Window

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```
>> [p,tbl,stats] = anova1(TSS2)

p =

  4.7831e-011


tbl =

    'Source'     'SS'          'df'    'MS'          'F'          'Prob>F'
    'Columns'    [850.9600]    [ 4]    [212.7400]    [62.9408]    [4.7831e-011]
    'Error'      [ 67.6000]    [20]    [  3.3800]          []             []
    'Total'      [918.5600]    [24]            []          []             []


stats =

    gnames: [5x1 char]
         n: [5 5 5 5 5]
    source: 'anova1'
     means: [25.8000 29.6000 33.2000 38 42.2000]
        df: 20
         s: 1.8385
```

Table 4.27: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on TSS performance cont.

```
>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -7.2794   -3.8000   -0.3206
    1.0000    3.0000  -10.8794   -7.4000   -3.9206
    1.0000    4.0000  -15.6794  -12.2000   -8.7206
    1.0000    5.0000  -19.8794  -16.4000  -12.9206
    2.0000    3.0000   -7.0794   -3.6000   -0.1206
    2.0000    4.0000  -11.8794   -8.4000   -4.9206
    2.0000    5.0000  -16.0794  -12.6000   -9.1206
    3.0000    4.0000   -8.2794   -4.8000   -1.3206
    3.0000    5.0000  -12.4794   -9.0000   -5.5206
    4.0000    5.0000   -7.6794   -4.2000   -0.7206

m =

   25.8000    0.8222
   29.6000    0.8222
   33.2000    0.8222
   38.0000    0.8222
   42.2000    0.8222

fx >>
```

4.7.3 Phase III results for the removal of nitrogen

In this Section, results for removal of ammonia-nitrogen and nitrate-nitrogen, are presented and discussed. Ammonia-nitrogen was monitored in the influent, RAS underflow and E-CLR. Nitrate was monitored in influent, E-ANX-C, E-AER-C, and E-CLR. Parameters such as nitrification efficiency, denitrification percentage, specific nitrification rate, specific denitrification rate, influent COD/N ratio, and COD/NO_3^-N ratio were evaluated from the measured concentration. Nitrite was measured in the effluent as quality criteria to ensure no inhibition to nitrification process.

4.7.3.1 Phase III results for removal of Ammonia-nitrogen

Nitrification in i-SGBR for phase III operation, assessment was based on the removal of ammonia nitrogen on nitrification efficiency. The evaluation was carried out between days 164 to 278. Steady state for each ammonia loading rate (ALR) was observed for the five stages operated; phase III-A, phase III-B, phase III-C, phase III-D and phase III-E. The profile of influent ammonia nitrogen and its serial progression to E-ANX-C, E-AER-C, RAS and effluent clarifier as final treatment stage is shown in Figure 4.66. It was observed that the average value and standard deviation for ammonia nitrogen concentration throughout the experiment was observed to be 52.6±2.2 mg/L. Maximum and minimum values for the influent ammonia nitrogen observed were 57 mg/L and 48.3 mg/L, respectively. Phase III was operated with ALR between 42.4±0.5 to 60.2 mg NH_3-N / m^3 d.

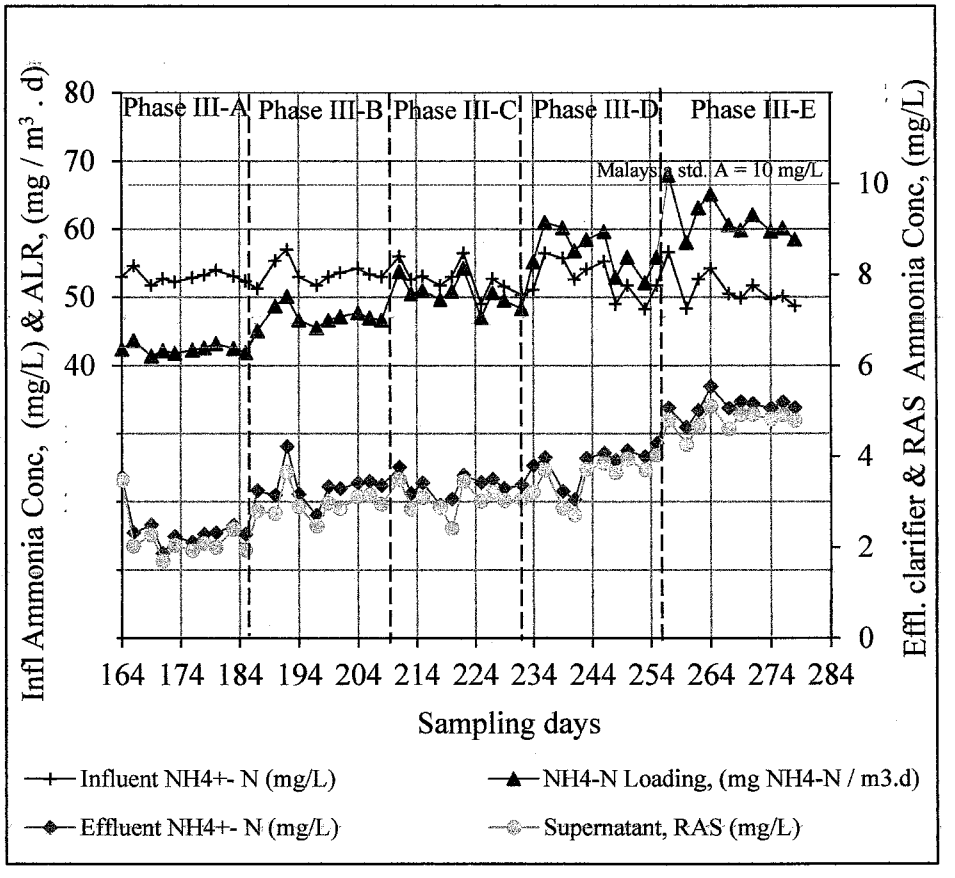

Figure 4.66: Infl., Ret. Activ. sludge, Effl.-Clarifer Ammonia-nitr. conc and Ammon. loading rate vs Days

Performance evaluation for the i-SGBR nitrification in phase III was assessed based on nitrification efficiencies (η_N) and nitrification rates (r_N). The interpretation of steady state values in Figure 4.66 is provided in Figure 4.67 with details of results for ammonia nitrogen in influent, E-CLR and Nitrification efficiency.

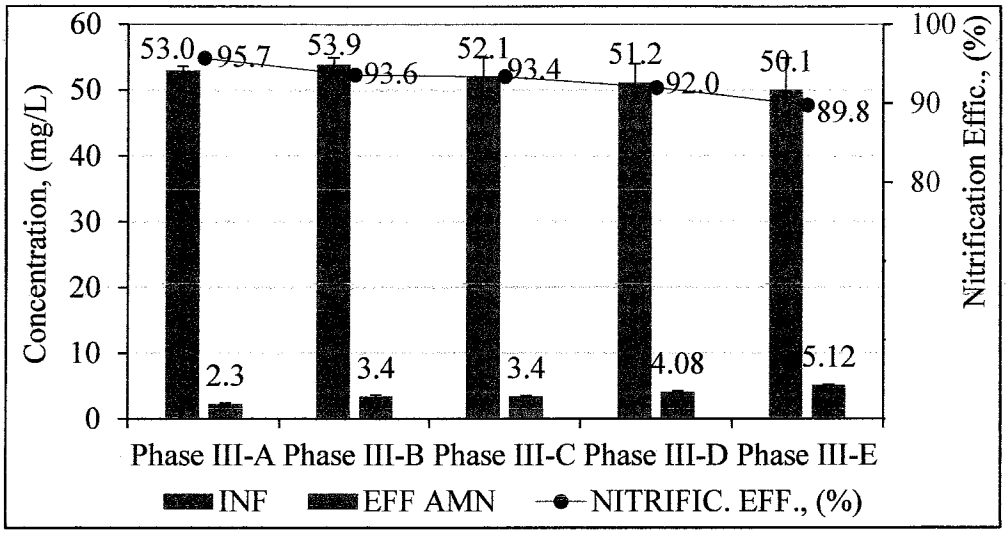

Figure 4.67: Results for steady state Influent & Effluent ammon. nitr. and Nitrif. Effic.

In phase III-A operation, the experiment was conducted between days 162 to 185, where the steady state was observed between days 173 to 185. Average value for the ALR applied to the system was observed to be 42.4±0.5 mg NH_3-N / m^3.d with equivalent influent average ammonia nitrogen concentration of 53±0.6 mg/L. The influent COD/N ratio of 11.8 was observed for phase III-A. The observed average value for E-CLR ammonia nitrogen concentration observed was 2.3±0.1 mg/L with observed η_N of 95.7±0.2 %. The r_N was observed to have average value and standard deviation of 6.2±0.5 mg NH_3-N/g. VSS. d. Ammonia nitrogen measured in the RAS underflow was observed to be slightly lower with an average value of 2.1±0.2 mg/L. This might be due to tendencies for the release of ammonia-nitrogen from organic nitrogen.

Phase III-B experiment was conducted between days 211 to 208, where steady state for the operation was observed between day 199 to day 208. At observed steady state, concentration for the E-CLR was observed to have average value and standard deviation of 3.4±0.2 mg/L. Similar phenomenon for an increase in ammonia nitrogen concentration, although insignificant, was observed from RAS ammonia nitrogen with

an average observed concentration of 2.1±0.2 mg/L. The same reason may be applicable as the previous as due to the large content of biomass in the clarifier, where organic nitrogen might be converted to ammonia nitrogen, thereby releasing ammonia nitrogen. The average value and standard deviation for ALR was observed to be 48.2±2.8 mg NH_3-N/m^3.d. The corresponding influent ammonia nitrogen concentration was observed to be 53.9±1.1 mg/L. The observed η_N was 93.6±0.2 %, and r_N was observed to have average value and standard deviation of 14.2±0.7 mg NH_3-N/g VSS. d. Corresponding influent COD/N ratio was observed to be 11.7.

In phase III-C operation, the experiment was conducted between days 222 to 232 with observed steady state achieved between days 173 to day 185. Average value for the ALR applied to the system was observed to be 50±2.7 mg NH_3-N/m^3.d with equivalent influent average ammonia nitrogen concentration of 52.1±2.9 mg/L. The influent COD/N ratio of 12.1 was observed. The observed average value for E-CLR ammonia nitrogen concentration observed was 3.4±0.1 mg/L with observed η_N of 93.4±0.3 %. The r_N was observed to have average value and standard deviation of 15±0.5 mg NH_3-N/g. VSS. d. Ammonia nitrogen measured in the RAS underflow was observed to be slightly lower with an average value of 3.2±0.2 mg/L, which could be due to the possibility for release of ammonia from organic nitrogen.

Phase III-D was operated between days 234 to 255, and its steady state was operated between day 246 to day 255. ALR of 55.3±3 mg NH_3-N / m^3.d was applied in the i-SGBR system with equivalent influent average ammonia nitrogen concentration of 51.2±2.8 mg/L. The influent. The influent COD/N ratio was observed to be 12.5. The average value and standard deviation for η_N was observed to be 92±0.4 % with subsequent observed E-CLR and RAS average concentration values and standard deviation of 4.08±0.14 mg/L and 3.83±0.16 mg/L, respectively. The rise of 6 % in ammonia nitrogen was observed from RAS which might be likely due to metabolism process for conversion of organic nitrogen to ammonia in the clarifier. The r_N was observed to have average value and standard deviation of 16.3±1 mg NH_3-N/g. VSS d.

In phase III-E operation carried out between days 257 to 278, its steady state was observed between day 269 to day 278. The average concentration of influent ammonia

nitrogen observed was 50.1±5 mg/L and its corresponding E-CLR effluent was observed to be 5.12 mg/L. The concentration of ammonia nitrogen at RAS was observed to be 4.8±0.1 mg/L as being slightly lower that E-CLR concentration. This gives average value and standard deviation for η_N as 89.8±0.2 %. ALR of 60.2±1.2 mg NH_3-N/m^3.d was applied in i-SGBR system at steady state, where r_N was observed with average value and standard deviation of 16.9±1 mg NH_3-N/g. VSS d.

A statistical analysis (ANOVA) was conducted on the effluent clarifier ammonia nitrogen average concentration values obtained from the experimental data. At 95 % confidence level, the result has indicated significant difference ($P<0.05$) between the mean values of the concentration at respective HRT's (Table 4.28)

Table 4.28: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on ammonia-nitrogen performance

Command Window

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```
>> [p,tbl,stats] = anova1(ammonia2)

p =

  7.9214e-013


tbl =

    'Source'     'SS'          'df'    'MS'          'F'             'Prob>F'
    'Columns'    [8.1591]      [ 3]    [2.7197]      [196.2443]      [7.9214e-013]
    'Error'      [0.2217]      [16]    [0.0139]            []                []
    'Total'      [8.3809]      [19]          []            []                []


stats =

    gnames: [4x1 char]
         n: [5 5 5 5]
    source: 'anova1'
     means: [2.2987 3.2224 3.4440 4.0800]
        df: 16
         s: 0.1177
```

Table 4.28: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on ammonia-nitrogen performance cont.

```
>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -1.1367   -0.9237   -0.7107
    1.0000    3.0000   -1.3584   -1.1453   -0.9323
    1.0000    4.0000   -1.9944   -1.7813   -1.5683
    2.0000    3.0000   -0.4346   -0.2216   -0.0086
    2.0000    4.0000   -1.0706   -0.8576   -0.6446
    3.0000    4.0000   -0.8490   -0.6360   -0.4230


m =

    2.2987    0.0526
    3.2224    0.0526
    3.4440    0.0526
    4.0800    0.0526
```

The results indicated that the mean value for HRT with flow rate of 100 L/d was lower than the mean values of other flow rates having 110, 120, 135 and 150 L/d. Hence, HRT with a flow rate of 100 L/d achieved better performance of nitrification performance compared to HRT with flow rates of 110, 120, 135 and 150 L/d.

4.7.3.2 Phase III results for removal of nitrate-nitrogen

Samples were collected from influent, E-ANX-C, E-AER-C (IR) and E-CLR to test for nitrate nitrogen. Nitrates into ANX-C was determined based on combined flow from influent, IR flow and RAS flow nitrate-nitrogen concentrations according to Equation 3.27. Denitrification process in the ANX-C was achieved through recycling of the nitrates formed in the AER-C during the nitrification process. Performance parameters such as denitrification efficiency (η_D), specific denitrification rate (r_D) and

$\Delta COD/\Delta NO_3$-N were evaluated from the measured nitrate concentration and MLVSS concentration in the ANX-C. Detailed analysis for the evaluation of parameters is provided in Appendix (Refer to Table E1.1 Appendix E). Figure 4.68 shows the time-dependent profile of E-AER-C, RAS, and E-CLR, while Figure 4.69 shows nitrates into ANX-C, denitrified E-ANX-C nitrate concentration and η_D. Interpretation for the steady state values for graphs in Figure 4.68 and Figure 4.69 is provided in Figure 4.70, with details of results for nitrate; influent, I-ANX-C, E-ANX-C, E-AER & E-CLR. The error bars represent standard deviation.

Internal recycle ratio (IR) of 6 was used throughout the experiment in phase III. IR ratio for phase III-A through phase III-E corresponds to IR flow of 600, 660, 720, 810 and 900 L/d. Influent nitrate was observed to be in the range of 0.1 mg/L to 0.6 mg/L, with an observed average of 0.4±0.2 mg/L. Influent nitrate nitrogen concentration was observed to be low. Although, this is possible due to nitrifier activity is not expected.

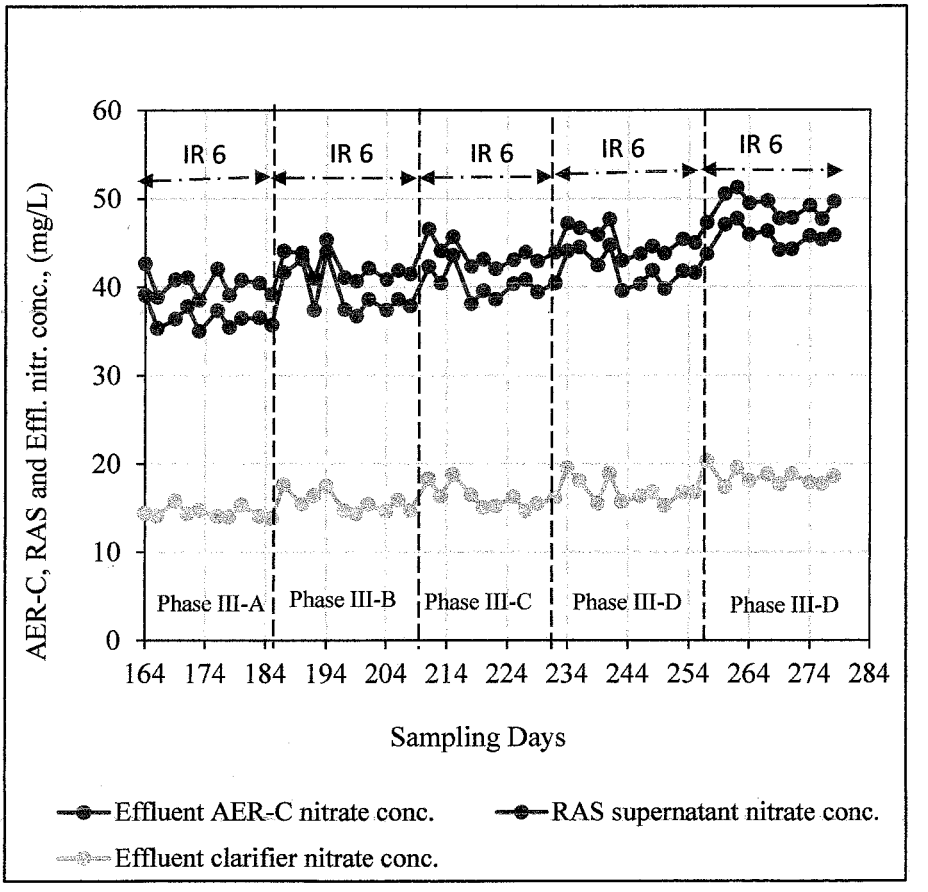

Figure 4.68: Aeration and Effl. nitrate conc. vs Sampling Days

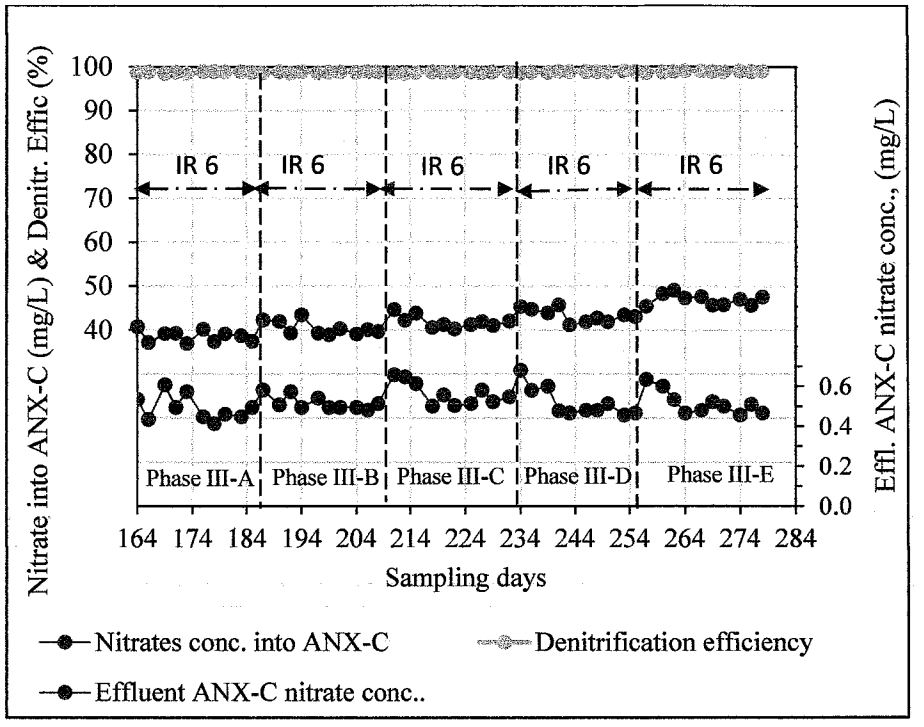

Figure 4.69: Nitr. into Anoxic and Effl. Anoxic chamber nitrate conc. vs Sampling Days

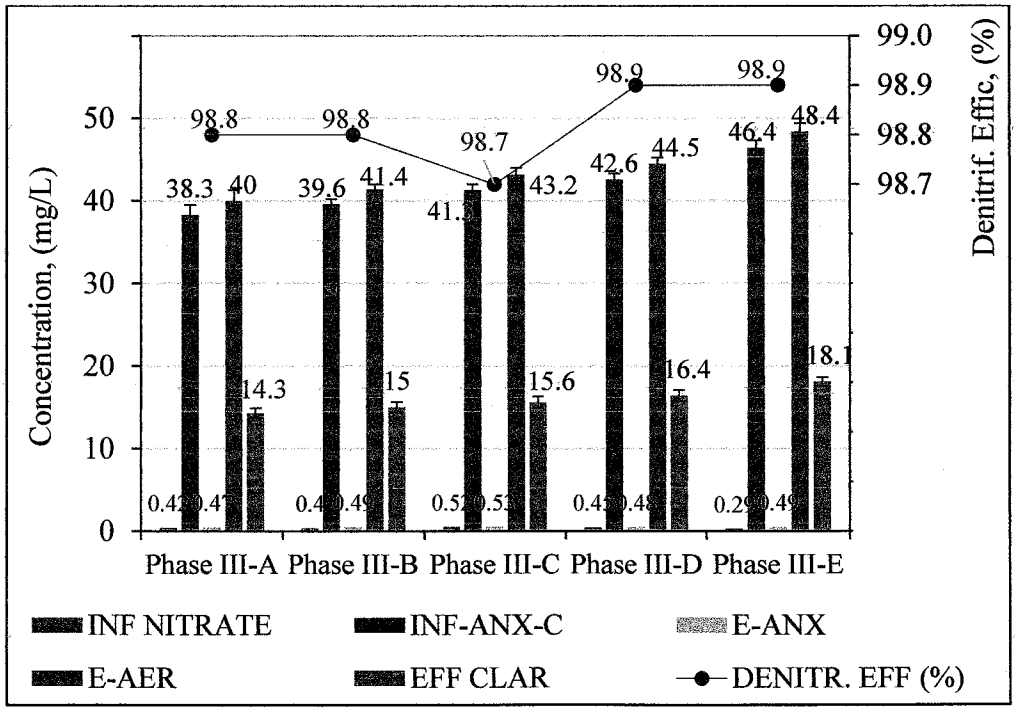

Figure 4.70: Results for nitrate; Infl., Infl.-Anoxic, Effl.-Anoxic, Effl.-Aeration & Effl.-Clarifier vs Phases

Between days 173 to 185, steady state was observed for phase III-A. The operation was carried out with nitrate loading into ANX-C observed to have average value and standard deviation of 0.62±0.02 g NO_3-N/m^3 d. The observed E-CLR nitrate nitrogen concentration of 14.3±0.6 mg/L was achieved. Consequently, influent and E-AER-C nitrate nitrogen concentration were observed as 0.42±0.3 and 40±1.3 mg/L, respectively. Nitrate into ANX-C and E-ANX-C were observed to be 38.3±1.2 and 0.47±0.06 mg/L, respectively. The η_D percentage was observed to be 98.8±0.2 %. The r_N was observed to be 288±17 mg NO_3-N/g VSS d. Specific COD removal rate of 0.72±0.02 mg COD/mg VSS d was determined in ANX-C. Similarly, the ANX-C $\Delta COD/\Delta NO_3$-N ratio was observed to be 10.8±0.36.

Between days 199 to 208, steady state was observed for phase III-B operation with observed nitrate loading rate into the ANX-C of 0.71±0.01 g NO_3-N/m^3 d. At the observed steady state, the following parameters were observed as average and standard deviation where applicable; E-CLR nitrate-nitrogen concentration of 15±0.6 mg/L, influent and E-AER-C nitrate nitrogen concentration 0.4±0.2 and 41.4±0.6 mg/L, respectively, nitrate nitrogen into ANX-C and E-ANX-C as 39.6±0.6 mg/L and 0.49±0.01 mg/L, respectively. The performance of η_D percentage and r_D were observed as 98.8±0.04 % and 98.7±0.09 mg NO_3-N/g VSS d, respectively. Specific COD removal rate and $\Delta COD/\Delta NO_3$-N ratio in ANX-C were observed as 0.39±0.04 mg COD/mg. VSS d and 10.2±0.9, respectively.

Between days 222 to 232, steady state was observed for phase III-C operation. The operation was carried out with nitrate loading into ANX-C observed with an average value and standard deviation of 0.82±0.01 g NO_3-N/m^3 d. The E-CLR nitrate-nitrogen concentration was observed with an average value and standard deviation of 15.6±0.7 mg/L. Corresponding influent and E-AER-C nitrate concentration were observed with average value and standard deviation of 0.52±0.1 and 43.2±0.8 mg/L, respectively. Concentration of nitrate into ANX-C and E-ANX-C were observed with average values and standard deviation of 41.3±0.7 and 0.53±0.03 mg/L, respectively. η_D percentage achieved was observed as 98.7±0.06 %. Similarly, the r_D was observed as 242±4.5 mg NO_3-N/g VSS d. Specific COD removal rate and $\Delta COD/\Delta NO_3$-N ratio

observed in ANX-C were observed with an average value and standard deviation of 0.29±0.03 mg COD/mg VSS d and 9.1±0.9, respectively.

Between days 246 to 255, steady state was observed for phase II-D, where nitrate loading rate was observed with an average value and standard deviation of 0.9±0.02 g NO_3-N/m^3 d. With the observed nitrate loading rate, the equivalent E-CLR nitrate-nitrogen concentration of 16.4±0.7 mg/L was observed. The corresponding influent and E-AER-C nitrate nitrogen concentration were observed with an average value and standard deviation of 0.45±0.1 mg/L and 44.5±0.7 mg/L, respectively. The observed concentration of nitrate nitrogen into ANX-C and E-ANX-C were 42.6±0.7 and 0.48±0.2 mg/L, respectively. The observed performance based on η_D percentage and r_D were observed as 98.9±0.07 % and 238.6±6 mg NO_3-N/g VSS .d. Subsequently, specific COD removal rate and $\Delta COD/\Delta NO_3$-N ratio for the denitrification process were observed with an average value and standard deviation of 0.27±0.02 mg COD/mg VSS .d and 8.6±0.5, respectively.

Between days 269 to 278 for the phase III-E operation, steady state was observed. The operation was observed with ANX-C nitrate loading having average value and standard deviation of 1.12±0.02 g NO_3-N/m^3d. Corresponding E-CLR nitrate-nitrogen concentration was observed with an average value and standard deviation of 18.1±0.5 mg/L. The corresponding influent and E-AER-C nitrate nitrogen concentration were observed with an average value and standard deviation as 0.29±0.2 and 48.4±0.9 mg/L, respectively. The observed concentration of nitrate nitrogen into ANX-C and E-ANX-C were observed with average values and standard deviation of 46.4±0.9 mg/L and 0.49±0.03 mg/L, respectively. The η_D percentage observed was 98.9±0.08 %. Similarly, r_D of 252±7.6 mg NO_3-N/g. VSS .d was observed. Specific COD removal rate and $\Delta COD/\Delta NO_3$-N ratio observed in ANX-C were observed with average value and standard deviation of 0.27±0.01 mg COD/mg. VSS .d and 8.1±0.3, respectively.

A statistical analysis (ANOVA) was conducted on the E-CLR nitrate-nitrogen results obtained from the experimental data. At 95% confidence level, the result has shown significant difference ($P<0.05$) exist between the mean values of concentration

at respective HRT's (Table 4.29 and Table 4.30), where the results indicated that the mean value for HRT with flow rate of 100 L/d was lower than the mean values of other flow rates having 110, 120, 135, and 150 L/d. Hence, HRT with a flow rate of 100 L/d achieved better performance of denitrification performance compared to HRT with flow rates of 110, 120, 135 and 150 L/d, respectively.

Table 4.29: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on overall system denitrification performance from E-Clarifier.

Command Window

New to MATLAB? Watch this Video, see Demos, or read Getting Started.

```
>> [p,tbl,stats] = anova1(nitrate4)

p =

  1.7875e-014


tbl =

    'Source'    'SS'          'df'    'MS'          'F'           'Prob>F'
    'Columns'   [59.8878]     [ 4]    [14.9720]     [145.1671]    [1.7875e-014]
    'Error'     [ 2.0627]     [20]    [ 0.1031]     []            []
    'Total'     [61.9505]     [24]    []            []            []


stats =

    gnames: [5x1 char]
         n: [5 5 5 5 5]
    source: 'anova1'
     means: [13.6660 14.6640 15.3900 16.5480 18.1400]
        df: 20
         s: 0.3211
```

Table 4:29: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on system overall denitrification performance cont.

```
>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -1.6058   -0.9980   -0.3902
    1.0000    3.0000   -2.3318   -1.7240   -1.1162
    1.0000    4.0000   -3.4898   -2.8820   -2.2742
    1.0000    5.0000   -5.0818   -4.4740   -3.8662
    2.0000    3.0000   -1.3338   -0.7260   -0.1182
    2.0000    4.0000   -2.4918   -1.8840   -1.2762
    2.0000    5.0000   -4.0838   -3.4760   -2.8682
    3.0000    4.0000   -1.7658   -1.1580   -0.5502
    3.0000    5.0000   -3.3578   -2.7500   -2.1422
    4.0000    5.0000   -2.1998   -1.5920   -0.9842


m =

   13.6660    0.1436
   14.6640    0.1436
   15.3900    0.1436
   16.5480    0.1436
   18.1400    0.1436

fx >>
```

Table 4.30: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on E-ANX-C nitrate-nitrogen performance

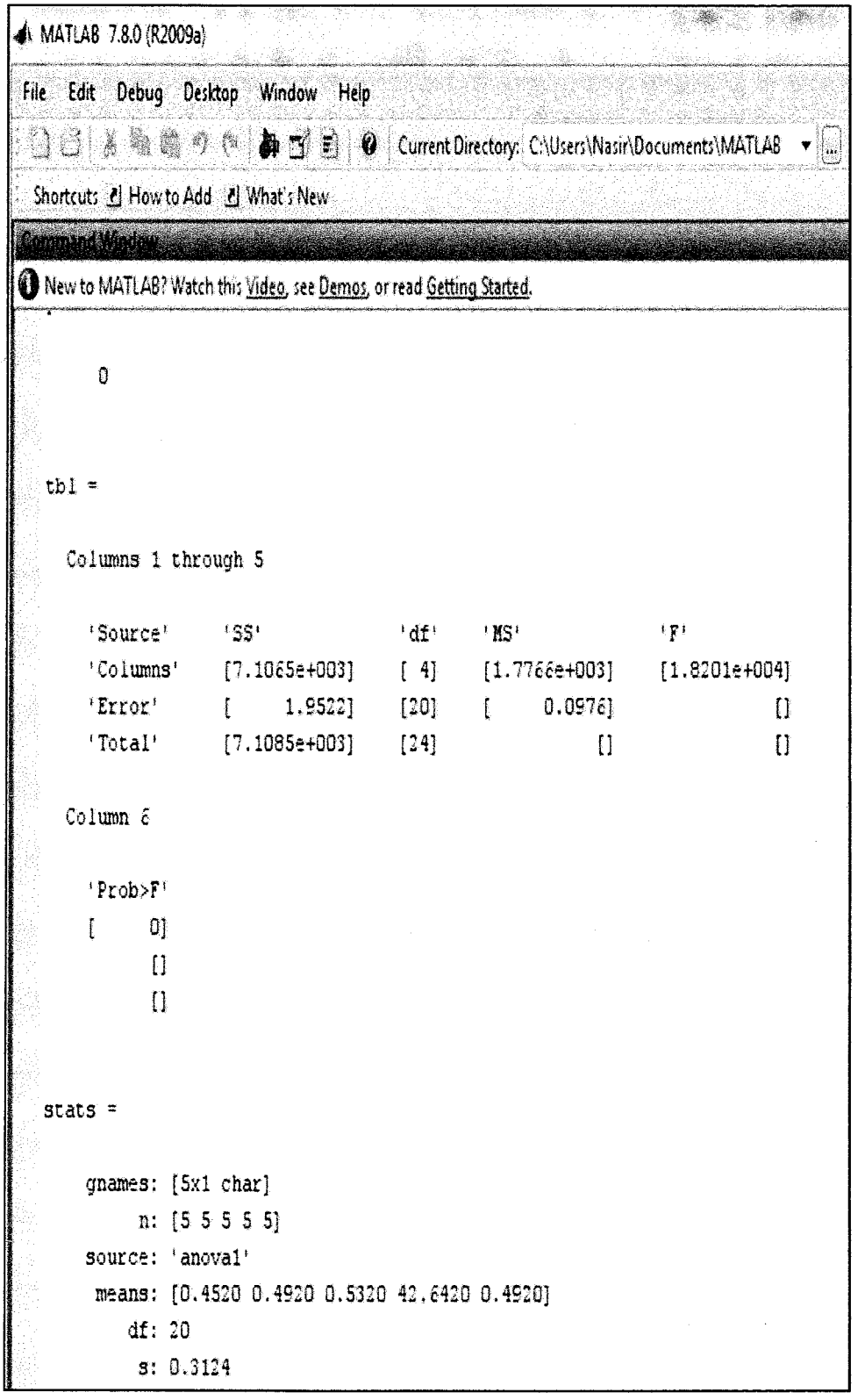

Table 4.30: Phase III-Statistical Analysis (ANOVA) and multiple comparison for effect of HRT on E-ANX-C nitrate-nitrogen performance cont.

```
>> [c,m] = multcompare(stats)

c =

    1.0000    2.0000   -0.6313   -0.0400    0.5513
    1.0000    3.0000   -0.6713   -0.0800    0.5113
    1.0000    4.0000  -42.7813  -42.1900  -41.5987
    1.0000    5.0000   -0.6313   -0.0400    0.5513
    2.0000    3.0000   -0.6313   -0.0400    0.5513
    2.0000    4.0000  -42.7413  -42.1500  -41.5587
    2.0000    5.0000   -0.5913         0    0.5913
    3.0000    4.0000  -42.7013  -42.1100  -41.5187
    3.0000    5.0000   -0.5513    0.0400    0.6313
    4.0000    5.0000   41.5587   42.1500   42.7413


m =

    0.4520    0.1397
    0.4920    0.1397
    0.5320    0.1397
   42.6420    0.1397
    0.4920    0.1397
```

4.7.3.3 Phase III results for removal of Total kjeldahl nitrogen and Total nitrogen

In phase III operation, wastewater samples were obtained in influent and effluent of the reactor to measure TKN and TN parameters. TKN comprises ammonia-nitrogen and organic nitrogen which was analysed by simplified TKN method. TN consist of both organic and inorganic forms of nitrogen. Since TN contains all nitrogen forms, the influent and effluent TN were compared with the composition of its constituents for the experimental phases, i.e phase III-A through phase III-E. The influent TN was compared with TKN, influent ammonia nitrogen and nitrate nitrogen as presented in Figure 4.71. Effluent TN was compared with effluent TKN, ammonia-nitrogen, nitrate

nitrogen and nitrite. Average effluent TN concentration and its constituents is presented in Figure 4.72. The error bars represent standard deviation of mean values observed.

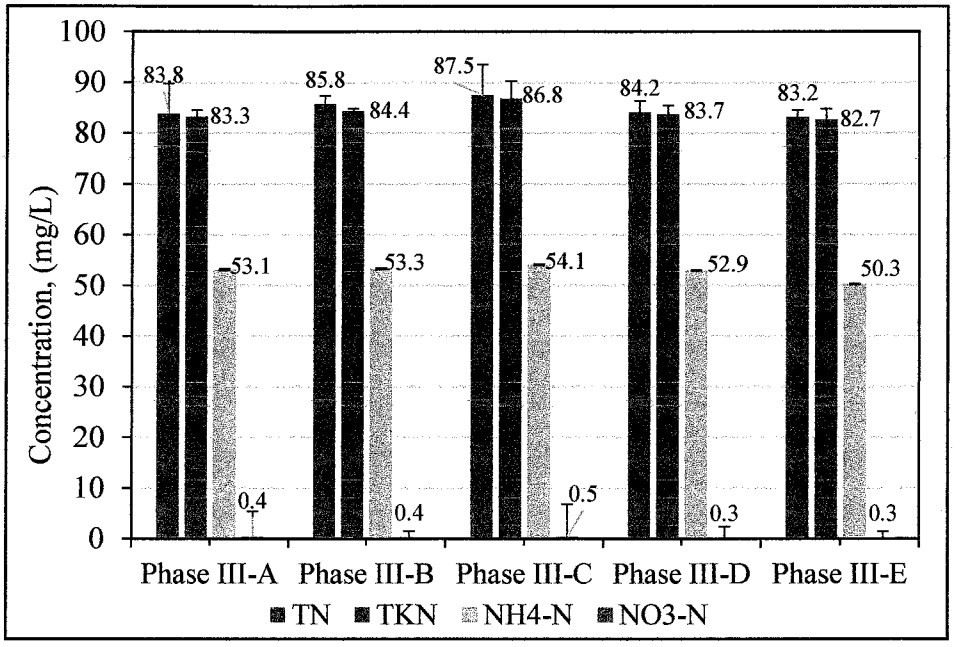

Figure 4.71: Profile of Total nitrogen conc. forms in influent vs Phases

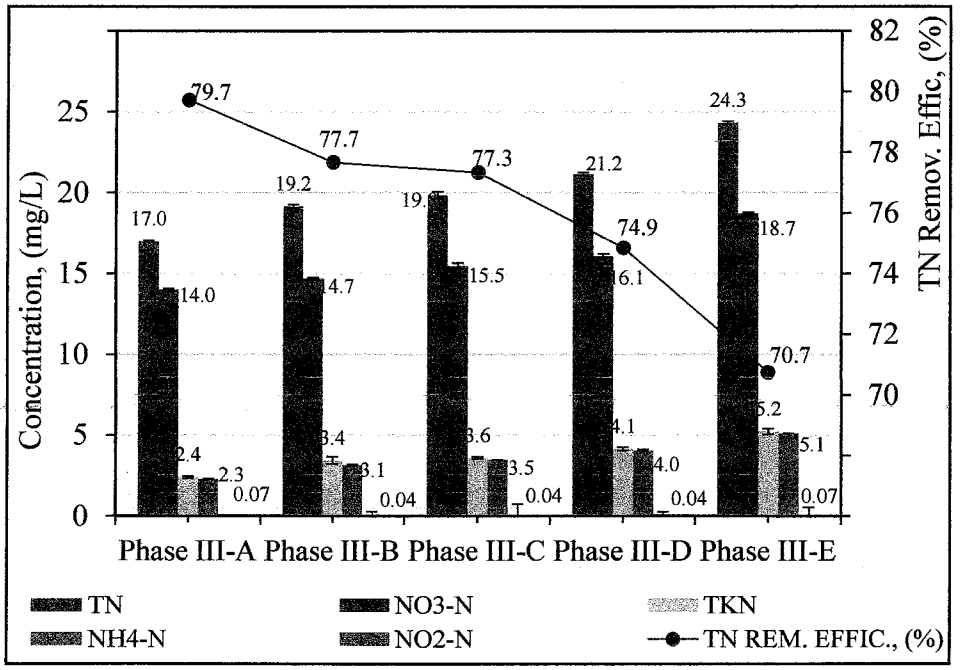

Figure 4.72: Profile of Total nitrogen conc. forms in effluent vs Phases

According to Figure 4.71, it was observed that influent TN and TKN were almost similar, however, differ by influent nitrate nitrogen concentration. Concentration of influent TN, TKN and nitrate nitrogen were observed to be in the range of 83.8-87.5 mg/L, 82.7-86.8 mg/L, and 0.3-0.5 mg/L, respectively. The low influent nitrate could be expected due to absence of nitrifiers. On the other hand, the concentration of influent ammonia nitrogen ranges from 50.3-54.1 mg/L, which when compared to TKN, influent organic nitrogen comprises between 37.7% (32.4 mg/L)-39.2 % (32.7 mg/L).

Conversely, the profile of effluent TN and its components such as TKN, ammonia nitrogen, nitrate and nitrite was observed with TN concentration in the range of 17 mg/L in phase III-A to 24.3 mg/L in phase III-E. The effect of HRT can be clearly seen with increase in effluent TN concentration from phase III-A operated with flow rate of 100 L/d through phase III-E operated with flow rate of 150 L/d. TN removal efficiency ranges between 79.7 % in phase III-A to 70.7 % in phase III-E. It was observed that substantial effluent TN concentration was contributed by effluent nitrate resulting from the oxidation of the organic matter in the AER-C. The residual effluent nitrate released could probably be due to lack of complete nitrate return to ANX-C for denitrification. Nitrate was observed to contribute between 70.7 % in phase III-A (14 mg/L) to 82.4 % (18.7 mg/L) in phase III-E of total effluent TN. TKN was observed to contribute between 14.1 % (2.4 mg/L) in phase III-A to 21.5 % (5.2 mg/L) in phase III-E. The concentrations of effluent TKN and ammonia nitrogen were observed to be similar, with equivalent effluent ammonia nitrogen concentration ranging from 2.3 mg/L in phase III-A to 5.1 mg/L in phase III-E. The similarity could be due to major oxidation of organic matter achieved in the AER-C, and probable conversion of organic nitrogen in TKN to ammonia nitrogen. The difference in concentration levels between ammonia nitrogen and TKN ranges between 0.1-0.3 mg/L. The effluent nitrite concentration measured was observed to be in the range of 0.04-0.07 mg/L, which probably was an indication of non inhibition of nitrification process. The effluent nitrite contribution ranges between 0.2-0.4 % which could be considered as insignificant.

4.7.3.4 Phase III results for summary of operation, control parameters, and performance data

In Phase III operation was observed between day 162 to day 278, five consecutive flow rates were operated in phases III-A (day 164-185), phase III-B (day 187 to 208), phase III-C (day 211-232), III-D (day 234-255) and phase III-E (day 257 to 278). These phases have operational flow rates corresponding to influent flow rates of 100, 110, 120, 135 and 150 L/d, respectively. Equivalent ANX-C and AER-C HRT for influent flow rates were 9.6 hrs, 8.7 hrs, 8.0 hrs, 7.1 hrs, 6.4 hrs, and 30 hrs, 27.3 hrs, 25 hrs, 22.2 hrs and 20 hrs, respectively. AD-C HRT of 1.00 day was operated for the entire phase. IR ratio was set to 6 corresponding IRQ flow of 900, 660, 720, 810, and 900 L/d for each operation in the phase, respectively. The values for the actual i-SGBR system SRT operated during the five stages in phase III were 20.0±0.39, 25.1±0.22, 30.8±1.05, 35.8±0.59, and 40.9±0.97 d, respectively. These SRT's operated were within the requirement for range of extended aeration.

The simultaneous aerobic digestion was operated with AD-C SRT of 10 days. The RAS ratio of 0.5 was set for operation during the five sub phases; phase III-A, III-B, III-C, III-D and III-E, corresponding to RAS flow rates of 50, 55, 60, 70 and 75 L/d, respectively. Corresponding i-SGBR system HRT during the five sub phases were 2.65 days, 2.41 days, 2.21 days, 1.96 days, and 1.77 days. The equivalent average and standard deviation values for ANX-C and AER-C OLR were (ANX-C:2.52±0.1, 2.75±0.05, 3.08±0.06, 3.50±0.07, 3.85±0.1 kg COD/m^3 d), and (AER-C: 0.49±0.01, 0.54±0.03, 0.62±0.03, 0.71±0.05, 0.79±0.04 kg COD/m^3 d), respectively.

In phase III-A, average values and standard deviation were observed in ANX-C, AER-C and IAD (RAS) as 3,362±69, 5010±65 and 13,055±360 mg/L, respectively. In phase III-B, average values and standard deviation MLSS concentration were observed in ANX-C, AER-C and IAD (RAS) as 3,514±72, 5,070±89 and 13,917±294 mg/L, respectively. In phase III-C, average values and standard deviation MLSS concentration were observed in ANX-C, AER-C and IAD (RAS) as 4,490±42, 5,166±151 and 15,622±270 mg/L, respectively. In phase III-D, average values and standard deviation MLSS concentration were observed in ANX-C, AER-C and IAD (RAS) as 5,215±74, 5212±119 and 18,107±169 mg/L, respectively. In phase III-E operation average values

and standard deviation MLSS concentrations in ANX-C, AER-C and IAD (RAS) were observed as 5,841±90, 5,237±92 and 19,475±236 mg/L, respectively.

There was an observed increasing trend in the system MLSS concentration in ANX-C, AER-C and RAS. This might be due to the increase in OLR operated over the period which could generate more volume of sludge to accumulate in the process. It was observed that reactor with higher COD loading achieved greater MLSS concentration, due incremental observed MLSS concentration, consequently, its effluent TSS was observed to be at higher level compared to the effluent TSS from reactor with lower loading rates. Summary of the phase III operational and performance results are presented; i-SGBR operational parameter (Table 4.31), pH, DO, and temperature (Table 4.32), performance data of COD, BOD_5, sCOD and TSS (Table 4.33) and performance data for TKN, TN, ammonia-nitrogen and nitrate (Table 4.34).

Table 4.31: Phase III results for summary of operational parameters

Phase	III				
Experimental stage	III-A	III-B	III-C	III-D	III-E
Experimental period (days)	164-185	185-208	208-232	232-255	255-278
Flow rate, Q_{INF}, (L/d)	100	110	120	135	150
Hydraulic retention time Θ_{ANX-C}, HRT (hours)	9.6	8.7	8.0	7.1	6.4
Hydraulic retention time Θ_{AER-C}, HRT (hours)	30	27.3	25.0	22.2	20.0
Hydraulic retention time Θ_{I-SGBR}, HRT (days)	2.65	2.41	2.21	1.96	1.77
Hydraulic retention time, aerobic digester, Θ_{AD-C}	24 hrs				
Hydraulic retention time, Clarifier, Θ_{CLAR} (days)	1.00	0.91	0.83	0.74	0.67
Organic loading rate, (OLR), ANX-C, (kg COD/m^3. d)	2.52±0.1	2.75±0.05	3.08±0.06	3.50±0.07	3.85±0.1
Organic loading rate, (OLR), AER-C, (kg COD/m^3. d)	0.49±0.01	0.54±0.03	0.62±0.03	0.71±0.05	0.79±0.04
F/M ratio for AER-C, kg COD/kg MLVSS. d	0.06±0.006	0.07±0.004	0.08±0.007	0.09±0.004	0.09±0.004
Solids retention time Θ_c, SRT_{I-SGBR} (days)	40.9±0.97	35.8±0.59	30.8±1.05	25.1±0.22	20.0±0.39
Solids retention time Θ_c, SRT_{AD-C} (days)	10				
Internal recycle flow, Q_{INF} *IR, (L/d)	600	660	720	810	900
Vol. Pumped in each cycle (12 cycles), (L)	50	55	60	68	75
Pumping duration, (minutes)	2.5	2.75	3	3.5	3.75
SVI, (mL/g)	76±1.3	71±2.6	62±1.8	55±1.28	50±1.6
MLSS in ANX-C, mg/L	3362±69	3514±72	4490±42	5215±74	5841±90
MLSS in AER-C, mg/L	5010±65	5070±89	5166±151	5212±119	5237±92
MLVSS in AER-C, mg/L	3021±88	3086±124	3104±142	3178±176	3228±112
MLSS IAD, mg/L	13,055±360	13,917±294	15,622±270	18,107±169	19,475±236
MLVSS IAD, mg/L	10,893±257	11,609±275	13,113±326	14,606±113	15,021±197
Working RAS ratio, Ri = [X/(Xr-X)]	0.5	0.5	0.5	0.5	0.5
Total RAS flow (R_{Total}) = (R_i) * Q_{INF}, (L/d)	50	55	60	70	75

Table 4.32: Phase III results for summary of pH, DO, and temperature

Experimental phase	Operational Period (days)	Parameter	Measured values			
			Chamber	Max.	Av. ±SD	Min.
III A-E	162-278	pH	INF	6.6	5.9±0.6	4.4
			ANX-C	7.7	7.5±0.1	7.2
			AER-C	7.6	7.5±0.1	7.2
		DO (mg/L)	ANX-C	0.28	0.24±0.02	0.21
			AER-C	4.89	4.45±0.26	3.98
		Temp. (°C)	AER-C	33.3	29.9±1.1	27.4

Table 4.33: Phase III results for steady state performance data of organic matter and TSS

Phase III	SSP	SP	**COD (mg/L)**	**sCOD (mg/L)**	**BOD_5 (mg/L)**	**TSS (mg/L)**	**Ratio**	
			Av.±SD	Av.±SD	Av.±SD	Av.±SD	BOD_5/ COD	COD/TSS
III-A	173-185	INF	1003±30	424±12	500±23	507±7	0.5	2.0
III-B	199-208		995±21	406±9	653±11	501±11	0.7	2.0
III-C	222-232		1028±25	443±15	607±52	503±25	0.6	2.0
III-D	246-255		1026±9	427±10	570±40	489±19	0.6	2.1
III-E	269-278		1020±15	407±16	507±193	464±6	0.5	2.2
III-A	173-185	E-ANX	591±10	232±1.7				
III-B	199-208		596±18	241±6.8				
III-C	222-232		657±33	254±13				
III-D	246-255		666±14	265±7				
III-E	269-278		650±11	278±2.3				
III-A	173-185	E-AER		20±2.0				
III-B	199-208			23±1.4				
III-C	222-232			25±2.4				
III-D	246-255			28±2.0				
III-E	269-278			33±0.8				
III-A	173-185	E-CLR	21±2	17±1	6.3±7	27±2	0.4	0.8
III-B	199-208		28±3	20±1	6.8±4	31±3	0.3	0.9
III-C	222-232		33±3	24±1	7.8±3	33±2	0.2	1.0
III-D	246-255		41±2	25±2	8.3±1	38±2	0.4	1.1
III-E	269-278		49±3	29±2	10±1	41±1.8	0.3	0.9

SSP = Steady state period, SP = Sampling point, INF = Influent, E-ANX = Effluent anoxic chamber, E-AER = Effluent aeration chamber, E-CLR=Effluent clarifier, COD = Total chemical oxygen demand, sCOD = Soluble chemical oxygen demand, BOD_5 = Biochemical oxygen demand, and TSS = Total suspended solids

Table 4.34: Phase III results for steady state performance data of Total kjeldahl nitrogen, Total nitrogen, ammonia-nitrogen and nitrate

Phase II	SSP	SP	TN (mg/L)	TKN (mg/L)	NH_3-N (mg/L)	NO_3-N (mg/L)	Ratio	
			Av.±SD	Av.±SD	Av.±SD	Av.±SD	COD/N	COD/NO_3
III-A	173-185	INF	85.0±2.6	83.3±5.9	53±0.6	0.4±0.3	11.8	
III-B	199-208		85.7±0.9	84.4±1.6	54±1.1	0.4±0.2	11.7	
III-C	222-232		84.0±5	86.8±6.1	52±2.1	0.5±0.1	12.1	
III-D	246-255		81.5±2	82.2±0	51±2.8	0.5±0.1	12.5	
III-E	269-278		80.8±1	82.7±1.4	50±2.8	0.3±0.2	12.7	
III-A	173-185	I-ANX				38±1.2		11±0.4
III-B	199-208					39±0.6		10±0.9
III-C	222-232					41±0.7		9±0.9
III-D	246-255					43±0.7		9±0.5
III-E	269-278					46±0.9		8±0.3
III-A	173-185	E-ANX				0.5±0.1		
III-B	199-208					0.5±0.01		
III-C	222-232					0.5±0.03		
III-D	246-255					0.5±0.2		
III-E	269-278					0.5±0.03		
III-A	173-185	E-AER				40±1.3		
III-B	199-208					41±0.6		
III-C	222-232					43±0.8		
III-D	246-255					45±0.7		
III-E	269-278					48±0.9		
III-A	173-185	RAS			2.1±0.2			
III-B	199-208				3.1±0.2			
III-C	222-232				3.2±0.2			
III-D	246-255				3.8±0.2			
III-E	269-278				4.8±0.1			
III-A	173-185	E-CLR	15.4±0.1	2.3±0.1	2.3±0.1	14±0.6		
III-B	199-208		17.6±0.1	3.4±0	3.4±0.2	15±0.6		
III-C	222-232		18.7±0.3	3.5±0.2	3.4±0.1	15.6±0.7		
III-D	246-255		20.3±0.4	4.2±0	4.1±0.1	16.4±0.7		
III-E	269-278		22.8±0.2	5.2±0.1	5.1±0.1	18±0.5		

INF =Influent, I-ANX = Influent anoxic chamber, E-ANX-C = Efluent anoxic chamber, E-AER = Effluent aeration, E-CLR = Effluent clarifier

4.7.4 Phase III results-Determination of bio-kinetic coefficients for carbon oxidation

In this study, attempts were made to evaluate the performance of i-SGBR system through determination of biokinetic coefficients; maximum specific growth rate (μ_{max}), half velocity coefficient (K_s), growth yield coefficient (Y), and decay coefficient (K_d) for heterotrophic carbon oxidizing bacteria. Essentially, kinetics are valuable to understand system performance and design biological wastewater treatment process. Data from the reactor operation was acquired to generate a relevant statistical relationship between the rate of cell growth and consumption of substrate. This experiment was done to evaluate the biodegradability of beverage industrial wastewater under aerobic conditions.

To achieve this purpose, sCOD samples were obtained from E-ANX-C and E-AER-C for the period between days 162 and 278 of the experimental duration. Sludge samples to determine MLSS and MLVSS were obtained from the AER-C and underflow (RAS). Determination of the biokinetic coefficients in a continuous flow pilot reactor can normally be achieved through operating the system with various SRT, HRT, and resultant OLR until each steady state prevails at specified stages in the process. In this study, five flow rates were used which were consistent with extended aeration HRT considering AER-C volume of 125 L. Steady state data were obtained for the sCOD from E-ANX-C and E-AER-C, whereas for the biomass samples were taken in the AER-C and underflow, which were used to determine MLSS and MLVSS concentration for the operation. The functional relationship between biomass in the AER-C and underflow (RAS) is required to establish daily sludge wasting to main the SRT for the system operation. Equation 3.5 described in Section 3.4.2.1 was used to determine the SRT at various sludge wastage (Qw), with known MLSS concentration, known volume of AER-C, and known RAS concentration (Xr). MLSS for each day was first of all measured from the reactor and determined in order to maintain the SRT of the system. Data for each stage in the phase was reported as averages and standard deviation.

4.7.4.1 Phase III results for soluble COD concentration in E-ANX-C and E-AER-C

Samples for sCOD concentration were obtained from E-ANX-C and effluent AER-C as part of essential parameters to evaluate the biokinetic coefficients for carbon oxidation. Conditions for the operation and experimental design during the biokinetic study is shown in Table 4.35, where the experiment was segmented in five stages stated as phase III-A through phase III-E.

Table 4.35: Phase III operational conditions for biokinetic study

Phase	III				
Experimental stage	III-A	III-B	III-C	III-D	III-E
Steady state period (days)	164-185	185-208	208-232	232-255	255-278
Flow rate, Q_{INF}, (L/d)	100	110	120	135	150
Hydraulic retention time Θ_{ANX-C}, HRT (hours)	9.6	8.7	8.0	7.1	6.4
Hydraulic retention time Θ_{AER-C}, HRT (hours)	30	27.3	25.0	22.2	20.0
Hydraulic retention time Θ_{i-SGBR}, HRT (days)	2.65	2.41	2.21	1.96	1.77
Hydraulic retention time, Clarifier, Θ_{CLAR} (days)	1.00	0.91	0.83	0.74	0.67

The sCOD data for each steady state observed in phase III-A, phase III-B, phase III-C, phase III-D and phase III-E is summarized in Table 4.37. The OLR for the AER-C is shown in Figure 4.73 and profile for the sCOD in the E-ANX-C and E-AER-C is shown in Figure 4.74.

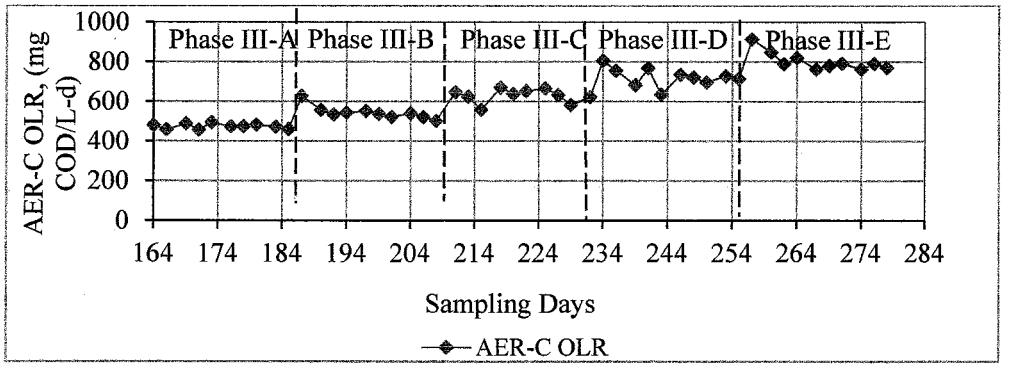

Figure 4.73: Aeration chamber organic loading rate vs Sampling Days

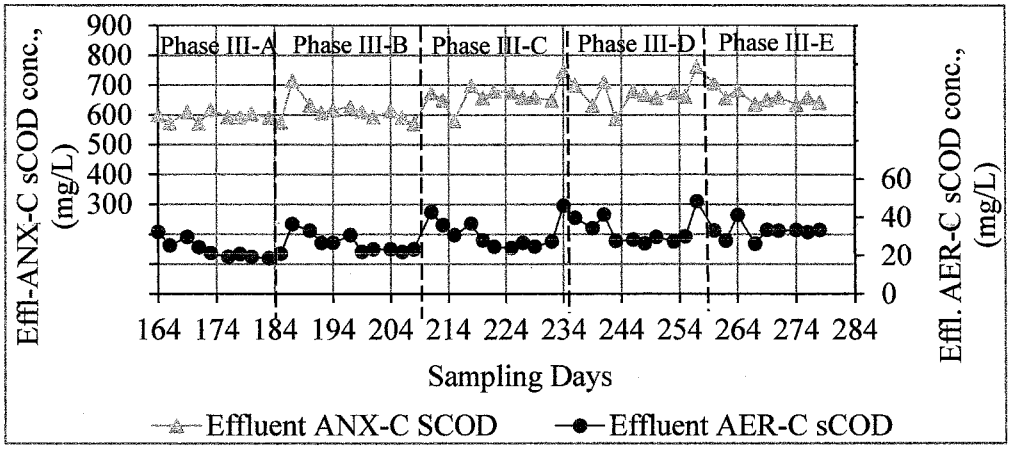

Figure 4.74: sCOD conc. Effl.-Anoxic and Effl.-Aeration chambers vs Sampling Days

4.7.4.2 Phase III results for MLSS and MLVSS profile in AER-C

Sludge samples were obtained from the AER-C (sample obtained from IR flow port) to determine its concentration required to evaluate necessary parameters for the biokinetic determination. The trend for AER-C MLSS and MLVSS for phase III operation is shown if Figure 4.75. The MLSS and MLVSS data for the AER-C for each steady state observed in phase III-A, phase III-B, phase III-C, phase III-D and phase III-E were summarized in Table 4.37. These stages were operated under different HRT, SRT, and OLR. Parameters such as MLSS in the AER-C was fixed and controlled throughout the duration of phase III.

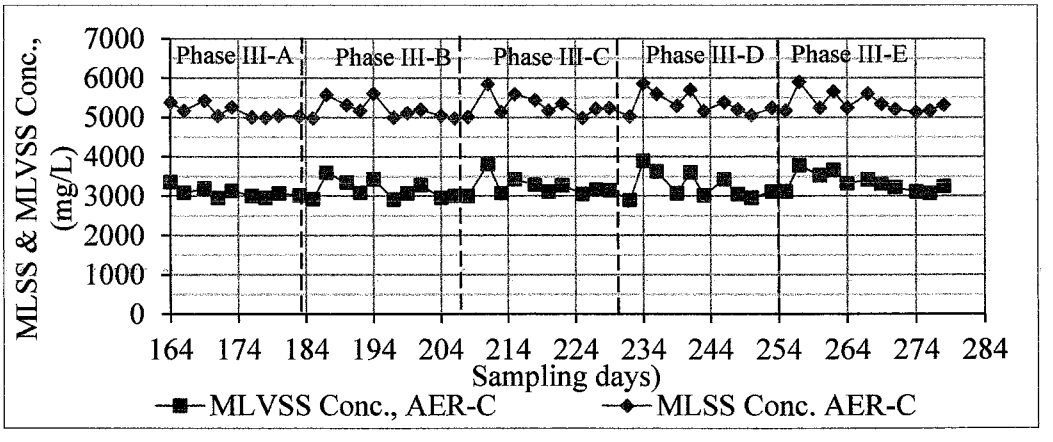

Figure 4.75: Aeration chamber MLSS and MLVSS conc. vs Sampling Days

4.7.4.3 Phase III results for MLSS and MLVSS profile in RAS

Figure 4.76 shows the underflow RAS concentration throughout the operation in phase III between days 162 to 278.

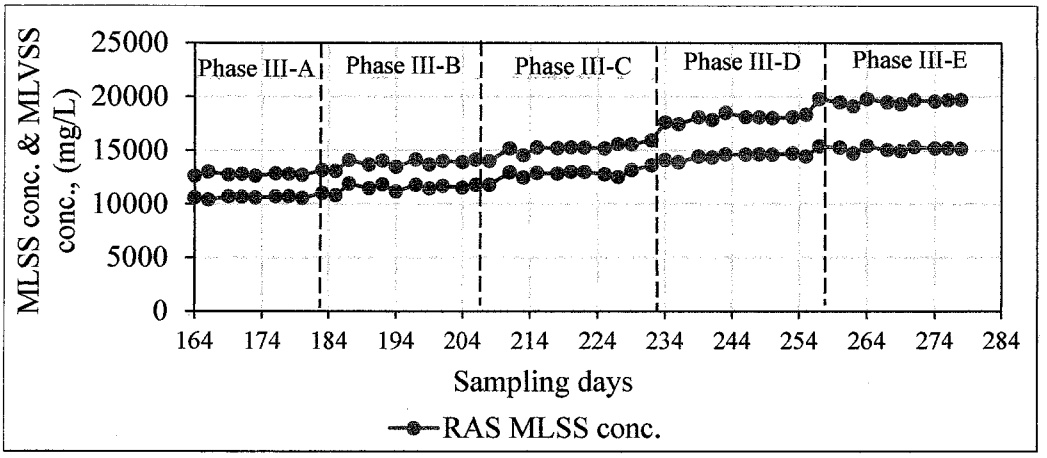

Figure 4.76: Return activated sludge MLSS and MLVSS conc. vs Sampling Days

The Return activated sludge concentration was determined as part of essential variables to maintain system SRT and retain the biomass concentration in the AER-C. Conversely, it can be seen from the physical trend in Figure 4.76, the accumulation of underflow concentration increases with OLR for successive stages in phase III operation. This could be due to the addition of organic matter into the system from a gradual increase in flow rate during the five successive stages of phase III-A through phase III-E. However, MLSS concentration in RAS between phase III-A operation and phase III-E was observed to range between average value and standard deviation of 13,055±360 and 19,475±236 mg/L. RAS is an important parameter in determining the amount of biomass concentration to be kept in AER-C, wasting and validation of RAS concentration from SVI. The summary of the RAS concentration data observed in phase III is detailed in Table 4.36.

Table 4.36: Phase III results for operational conditions of biokinetic study

Phase	III				
Experimental stage	III-A	III-B	III-C	III-D	III-E
Steady state period (days)	164-185	185-208	208-232	232-255	255-278
Flow rate, Q_{INF}, (L/d)	100	110	120	135	150
Hydraulic retention time Θ_{ANX-C}, HRT (hours)	9.6	8.7	8.0	7.1	6.4
Hydraulic retention time Θ_{AER-C}, HRT (hours)	30	27.3	25.0	22.2	20.0
Hydraulic retention time Θ_{I-SGBR}, HRT (days)	2.65	2.41	2.21	1.96	1.77
Hydraulic retention time, Clarifier, Θ_{CLAR} (days)	1.00	0.91	0.83	0.74	0.67
sCOD E-ANX-C, So (mg/L)	232±1.7	241±6.8	254±7.8	265±7.2	278±2.3
sCOD E-AER-C, Se (mg/L)	20±2	23±1.4	25±2.8	28±2.1	33±0.8
Organic loading rate, (OLR), AER-C, (kg COD/m^3. d)	0.49±0.01	0.54±0.03	0.62±0.03	0.71±0.05	0.79±0.04
F/M ratio for AER-C, kg sCOD/m^3. d	0.06±0.006	0.07±0.004	0.08±0.007	0.09±0.004	0.09±0.003
Solids retention time Θ_c, SRT_{I-SGBR} (days)	40.8±0.7	35.8±0.5	30.8±0.8	25.1±0.7	20.0±0.3
Internal recycle flow, Q_{INF} *IR, (L/d)	600	660	720	810	900
MLSS in AER-C, mg/L	5010±65	5069±89	5166±151	5212±119	5237±92
MLVSS in AER-C, mg/L	3021±88	3086±124	3104±142	3178±176	3228±112
MLSS in RAS, mg/L	13,055±360	13,917±294	15,622±270	18,107±169	19,475±236
MLVSS in RAS, mg/L	10,893±257	11,609±275	13,113±326	14,606±113	15,021±197
MLVSS/MLSS ratio AER-C	0.60	0.60	0.60	0.60	0.61
MLVSS/MLSS ratio RAS	0.85	0.83	0.84	0.81	0.77
Working RAS ratio, Ri = [X/(Xr-X)]	0.5	0.5	0.5	0.5	0.5
Total RAS flow (R_{Total}) = (R_i) * Q_{INF}, (L/d)	50	55	60	70	75

In phase III-A operation, steady state was observed between days 173 to 185. Influent flow rate was set to 100 L/d, RAS flow of 50 L/d (RAS ratio = 0.5Q) and IRQ of 600 L/d (IR ratio = 6). The ANX-C and AER-C HRT operated were 9.6 hours and 30 hours, respectively. E-ANX-C and E-AER-C sCOD concentrations were observed as 232±5.7 and 20±5.3 mg/L, respectively. OLR in the AER-C based on total COD was observed as 0.49±0.01 kg COD/m^3. d. F/M in the AER-C was observed to have an average value and standard deviation of 0.06±0.01 kg sCOD/kg MLVSS. d. SRT operated for the period was observed to have an average value and standard deviation of 40.8±0.7 days. Average values and standard deviation for MLSS and MLVSS concentration in AER-C were observed to be 5,010±65 and 3,021±88 mg/L, respectively. This gives MLVSS/MLSS ratio of 0.6. The ratio was observed to be low, however, MLVSS/MLSS ratio for extended aeration was given in the range of 0.6 to 0.75 [332]. MLSS concentration in RAS was observed to have an average value and standard deviation of 13,055±360 mg/L. The operation was performed in a suspended growth mode during the entire period of the study. The values obtained in this present work have suggested the low supply of soluble substrate offer to the microorganisms, which could be due to the extended aeration and low organic loading. These F/M ratios are possible due to relatively high MLSS and MLVSS concentration level maintained for extended aeration as compared to other ASP, normally operated with MLSS levels below 2000 mg/L [85]. The pool of high biomass concentration and complete retention of solids all combined could make the process to be operated at low F/M ratio.

In phase III-B, steady state was observed between days 199 to 208. The operation was carried out with an influent flow rate of 110 L/d, RAS flow of 55 L/d (RAS ratio = 0.5) and IRQ of 660 L/d (IR ratio = 6). i-SGBR system SRT was observed to have average value and standard deviation of 35.8±0.59. Average values and standard deviation for MLSS and MLVSS concentration in AER-C were observed as 5069±89 mg/L and 3086±126 mg/L, respectively. This gives MLVSS/MLSS ratio of 0.6. RAS MLSS concentration was observed to have average value and standard deviation of

13,917±294 mg/L. HRT for ANX-C and AER-C were 8.7 and 27.3 hrs, respectively. The average OLR operated for AER-C was observed as 0.54±0.03 kg COD/m^3 d. F/M ratio in AER-C was observed as 0.07±0.004 kg COD/kg MLVSS d. Average and standard deviation sCOD concentrations observed for the E-ANX-C and AER-C were 241±6.8 and 23±1.4 mg/L, respectively.

In phase III-C, steady state for the operation was observed between day 222 to day 232. Influent flow rate of 120 L/d, RAS flow of 60 L/d (RAS ratio = 0.5), IRQ of 720 L/d (IR ratio = 6) were set as operational parameters. i-SGBR system SRT was observed to have average value and standard deviation of 30.8±1.05 days. i-SGBR system HRT operated was observed to be 2.21 days. The corresponding HRT in ANX-C and AER-C were observed to be 8 hrs and 25 hrs, respectively. The average OLR operated for AER-C was observed to be 0.62±0.03 kg COD/m^3.d. Average and standard deviation sCOD concentrations observed for the E-ANX-C and AER-C were 254±7.8 mg/L and 25±2.8 mg/L, respectively. *F/M* ratio determined in AER-C was observed as 0.08±0.01 kg COD/kg MLVSS d. The average and standard deviation values for MLSS and MLVSS concentration in AER-C was observed to be 5166±151 mg/L and 3104±140 mg/L, respectively. This gives MLVSS/MLSS ratio of 0.6. RAS MLSS concentration was observed to be 15,622±270 mg/L.

In phase III-D operation, steady state was observed between days 246 to day 255. Influent flow rate was fixed for 135 L/d, RAS flow of 70 L/d (RAS ratio = 0.5Q) and IRQ of 810 L/d (IR ratio = 6). ANX-C and AER-C HRT operated were 9.6 hours and 30 hours, respectively. The equivalent OLR in the AER-C based on total COD was observed to be 0.71±0.05 kg COD/m^3. d. *F/M* in the AER-C was observed have an average value and standard deviation of 0.09±0.004 kg sCOD/kg MLVSS d. The SRT operated for the period was observed to have an average value and standard deviation of 25.1±0.22 days. Average and standard deviation values for MLSS and MLVSS concentration in AER-C were observed as 5212±119 and 3179±176 mg/L, respectively. This gives MLVSS/MLSS ratio of 0.6. The RAS MLSS concentration was observed to have an average value and standard

deviation of 18,107±169 mg/L. E-ANX-C and E-AER-C sCOD concentrations were observed as 265±7.7 mg/L and 28±3.2, respectively.

In phase III-E operation, steady state was observed between days 269 to 274. Parameters such as influent flow rate were set at 150 L/d, RAS flow at 75 L/d and IRQ at 900 L/d. System SRT for i-SGBR was observed with an average value and standard deviation of 20.0±0.39 days. System HRT for i-SGBR operated was observed as 2.21 days. Corresponding HRT in ANX-C and AER-C were observed to be 6.4 hrs and 20 hrs, respectively. Average OLR operated for AER-C was observed with a value of 0.79±0.03 kg COD/m^3 d. Average and standard deviation sCOD concentrations observed in E-ANX-C and AER-C were 278±5.1 and 33±2.6 mg/L, respectively. F/M ratio was determined in AER-C with observed value of 0.09±0.004 kg COD/kg MLVSS d. The average and standard deviation values for MLSS and MLVSS concentration in AER-C were observed as 5,237±92 mg/L and 3,228±99 mg/L, respectively. This gives MLVSS/MLSS ratio of 0.61. RAS MLSS concentration was observed as 19,475±236 mg/L.

Linearized model was obtained by linear regression plots, where the reciprocal values of the specific substrate utilization rate (*1/U*) were plotted against reciprocal values for the E-AER-C sCOD concentration (*1/S*), hence, evaluation of the substrate removal kinetics was done according to Equation 3.11 in Section 4.5. The slope of the straight line yields *Ks/K*, while *1/k* was the intercept. The values of *Ks* and *k* were expressed in (mg sCOD/L) and hr^{-1}, respectively. Conversely, reciprocal values for the SRT (*1/Θc*) were plotted against the specific substrate utilization rate, *U* (mg sCOD/mg VSS. d) according to Equation 3.12 in Section 4.5, where the yield coefficient, *Y* (mg VSS/mg sCOD) was determined from the slope of the straight line, and the endogenous decay coefficient (K_d) was obtained from the intercept. K_d is measured as day^{-1}. The maximum specific growth rate, μ_{max} (d^{-1}) was determined by multiplying coefficients *k* and *Y* according to Equation 3.13 in Section 4.5.

The continuous flow data are generated for the period between days 162 and 278 is summarized in Table 4.37. This data was used to evaluate the kinetic coefficients. Equations 3.11 and Equation 3.22 can be applied in the case of a system with recycle or non-recycle of sludge [303, 304]. Figure 4.75 shows data plotted based on Equation 3.13, in which the relationship between SRT and utilization of the soluble organic matter in wastewater represented. In Figure 4.77, it can be seen that the model showed the coefficient of determination R^2 was 0.98 at 95 % level of significance, which is > 0.5. The model presents fitted data with good correlation. Based on the observed data, decay coefficient (K_d) and biomass yield (Y) were found to be -0.0172 d^{-1} and 0.72 mg VSS/mg sCOD, respectively. Whereas, in Figure 4.78, the linearized model was according to Equation 3.14 and experimental data was fitted into the model. The value for coefficient of determination (R^2) was observed as $R^2 = 0.99$ and presents a good correlation. The rate constant (k) and Monod's constant (K_s) were obtained as 2.81 d^{-1} and 979 mg sCOD/L, respectively. The maximum specific growth rate, μmax (d^{-1}) was determined as a product of coefficients k and Y based on Equation 3.13 in Section 3.5 with value observed as 2.03 d^{-1}.

Table 4.37: Data for determination of biokinetic coefficients.

Steady state periods in experimental phases III-A to III-E	i-SGBR data						
	Qo	*So*	*Se*	*Θc*	X_{MLVSS}	X_{MLSS}	*HRT,Θ*
	Lpd	mg/L	mg/L	days	mg/L	mg/L	hours
173-185	100	232±1.7	20±2.0	40.9±0.7	3021±88	5010±65	1.25
199-208	110	241±6.8	23±1.4	35.8±0.5	3086±124	5070±89	1.14
222-232	120	254±7.8	25±2.8	30.7±0.8	3104±89	5166±151	1.04
246-245	135	266±7.2	28±2.1	25.1±0.72	3178±176	5212±119	0.93
269-278	150	278±2.3	33±2.1	20.0±0.3	3228±112	5237±92	0.83

Computed data

Exp. phase	*(So-Se)* mg/L	*Θ* day	*XΘ* mg/L-d	*U =XΘ/So-Se* day	*1/Se* mg/L^{-1}	*1/U = So-Se/XΘ* d^{-1}	*1/Θc* d^{-1}
Phase III-A	224	1.25	3776	18	0.051	0.056	0.024
Phase III-B	213	1.14	3507	16	0.044	0.062	0.028
Phase III-C	235	1.04	3223	14	0.039	0.071	0.033
Phase III-D	229	0.93	2943	13	0.035	0.081	0.040
Phase III-E	228	0.83	2690	11	0.030	0.091	0.050

Qo = Flow rate (L/d), So = Influent AER-C sCOD conc (mg/L), Se = E-AER-C sCOD conc (mg/L), Θc = Solid retention time (days), X_{MLVSS} = Mixed liquor volatile susp. Solids conc (mg/L), X_{MLSS} = Mixed liqour suspended Solids (mg/L), Θ = Hydraulic retention time (hours or days), and U = Specific growth rate (d^{-1})

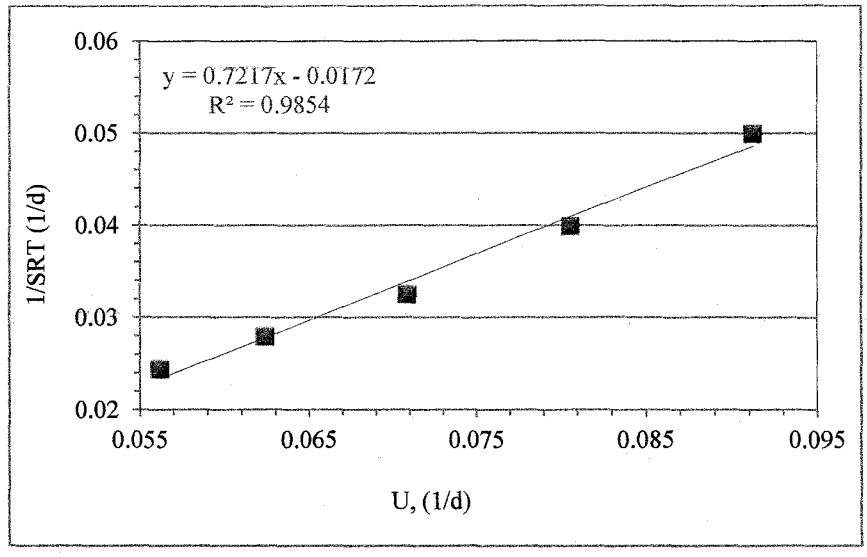

Figure 4.77: Graph of *U* vs. *1/SRT* on sCOD basis

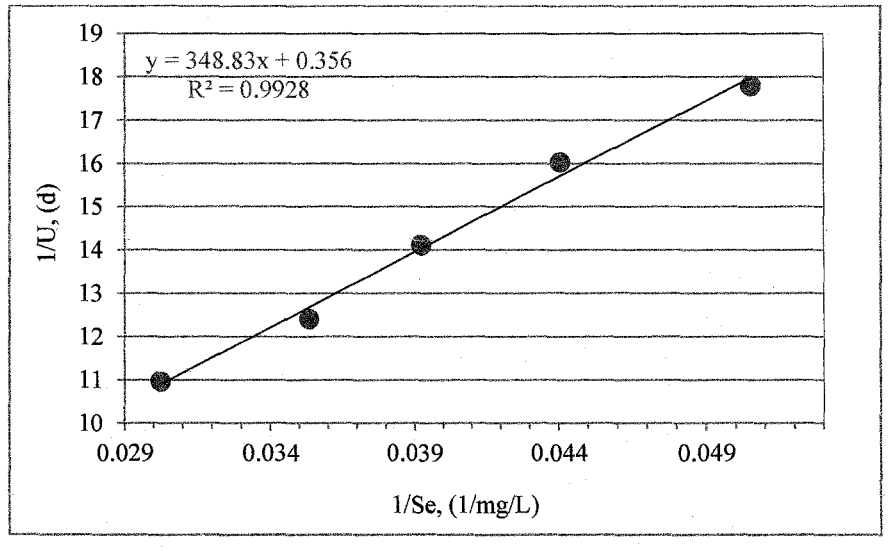

Figure 4.78: Graph of *1/S* vs. *1/U* on sCOD basis

Previous researches have studied biokinetic coefficients for ASP using different sources of wastewater (see Table 4.38). Values of bio-kinetic coefficients found experimentally and used to design biological treatment processes using ASP are also discussed elsewhere [85, 113].

Table 4.38: ASP kinetic coefficients reported for various organic sources (COD basis)

Substrate source	Y (mg VSS/mg sCOD)	K_d (d^{-1})	k (d^{-1})	Ks (mg sCOD/L)	μmax (d^{-1})	Ref.
Municipal sewage	0.2–0.5	0.03-0.07	2.0-8.0	20-80	0.4-4.0	[336]
Municipal sewage	0.4–0.8	0.025-0.075	0.8-8.0	15-70	2-10	[84]
Municipal sewage	0.31–0.35	0.016–0.068	0.5-0.6	43-223	1.7	[337]
Synthetic wastewater	0.49–0.58	0.037-0.151	0.63-3.75	289-293	1.28-6.46	[268]
Synthetic wastewater	0.42–0.53	0.05-0.19	0.34-3.34	83-646	0.8-6.3	[268]
Municipal sewage	0.62-1.25	0.0198-0.0308	2.54-3.16	311.7- 508	1.96-3.17	[280]
Food and beverage industry wastewater	0.2384	0.01	-	15.22	2.94	[277]
High oil and grease rendering wastewater	1.08-0.85	0.2-0.66	1-1.3	5580-5600	-	[273]
Beverage industry wastewater	0.72	0.0172	2.81	979	2.03	This study

It can be observed from Table 4.38 that values obtained for biokinetic coefficients in this study were; k as 2.81 d^{-1} and μ_{max} as 2.03 d^{-1}. These values are comparatively within normal range of reported values for these coefficients in ASP, they also vary relatively from one another to those reported in literature. The yield coefficient Y obtained as 0.72 mg VSS/mg sCOD d is similarly within range of coefficients reported for ASP. The decay coefficient, K_d, observed in this study as 0.0172 d^{-1} is also within range of values reported for some ASP. However, half velocity constant, Ks, with value of 979 mg COD/L was observed to be relatively high. This could possibly mean that the wastewater may be purely of organic nature. According to Raj et al. [317], the half velocity Ks for industrial wastewater generally vary between 850 to 5200 mg sCOD/L [338]. Mardini et al. [280] performed biokinetic study on extended aeration process with aeration tank MLSS of 5,000 mg/L. The results for k, Ks, Y and K_d were observed as 2.53 d^{-1}, 508 mg sCOD/L, 1.25 mg/mg and 0.198 d^{-1}, respectively. Mardani et al. [280] observed values such as Ks and Y were out of range of values compared to values reported for conventional activated sludge processes in literature. The study established difference was attributted with fact that, determining Ks value was affected by estimation of K_d, hence any uncertainty in estimating K_d will be reflected on corresponding Ks value. It was also observed that Y values were increasing with increase in MLSS concentration. The reason concluded on the Y value was attributed to nature of substrate and environment of the microbial medium, which could have significance to effect changes.

It can be observed that present study was conducted under relatively high MLSS concentration of extended detention times, with SRT between 20 to 40 days and HRT between 20 to 30 hours. Variation in the values of kinetic coefficients as compared to other ASP processes has been observed without major disparity from other experiments (Table 4.38). Although, Ks value was relatively higher than those observed in previous studies, however, it has been recognized that Ks could tend to vary with temperature and the nature of the substrate [192, 339]. In this study, the average temperature operated was 29.9±1.1 °C, which was mesophilic temperature range.

F/M ratio (COD basis) as a substrate offer to microorganisms was generally observed to be low from phase III-A through phase III-E, which could probably be due to substrate requirement in the reduction for denitrification and endogenous process in AER-C. F/M ratio during the period

ranges between 0.06–0.09 kg sCOD/kg MLVSS d. In extended aeration ASP, F/M ratio with range of 0.2–0.5 kg COD/kg MLVSS d on COD basis (equivalent to 0.05–0.15 kg BOD_5/kg MLVSS d on BOD_5 basis) have been reported [84, 333]. Values obtained in this present work have suggested the low supply of soluble substrate offer to the microorganisms, which could be due to the extended aeration and low organic loading to AER-C. The carbon requirement in ANX-C could also reduce the COD in the AER-C. Low F/M ratio was possible due to relatively high MLSS and MLVSS concentration within the range of 5,010 mg/L operated in phase III-A to 5237 mg/L operated in phase III-E. Extended aeration is operated with higher MLSS compared to other ASP such as high rate, which is usually operated with MLSS levels below 2000 mg/L [85]. The pool of high biomass concentration and complete retention of solids together could make the process operate under low F/M ratio. OLR gradually increased from 0.49 kg COD/m^3 d in phase III-A to 0.79 kg COD/m^3 d in phase III-E. It can be observed that the sCOD removal efficiency of 91. 5 % observed in phase III-A reduced to 88 % in phase III-E. This could be due to increase in the OLR and reduction of system HRT from 2.65 days to 1.77 days.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Chapter overview

This chapter provides a synopsis on the realization of the study objectives. The chapter reaffirms the research statement and discusses the study's findings. The research's conceptual and methodological contributions are discussed in this chapter. Finally, some suggestions are put forward for future research.

5.2 Conclusions

Present research was conducted for 278 days as pilot plant. The objectives were to design, evaluate performance of a continuous flow integrated bioreactor system on influence of various HRT and OLR, determination of heterotropic microbial interaction with substrate utilization, and determination of excess sludge degradation in the aerobic digestion chamber from food and beverage industry wastewater. The following conclusions can be made from research findings of the present work:

Design and development was made to integrate treatment units comprising pre-anoxic chamber, aeration chamber, aerobic digestion chamber, and clarifier into a single bioreactor system to treat food and beverage industry wastewater using suspended growth. Fabrication of the integrated suspended growth bioreactor system (i-SGBR) was achieved concentrically with common wall construction of all treatment chambers. Compact bioreactor was integrated to have first treatment unit as anoxic chamber with volumetric capacity of 40 liters, followed by aeration chamber with volumetric capacity of 125 liters. Aeration chamber effluent settles in final clarifier had

volumetric capacity of 100 L. The clarifier has achieved effective settlement of sludge formed in reaction chambers with clarified wastewater supernatant discharged. Aerobic digestion chamber was in between aeration chamber and final clarifier, although its operation was independent. The aerobic digestion chamber was fabricated with volumetric capacity of 75 liters. The bioreactor system was fitted with user friendly automated control systems, regulating the pumps, pH sensor, mixing devices, and compressed air.

Treatment efficiencies under different operating conditions (OLR, HRT, and IR) were studied based on bioreactor performance. Where in phase II, ANX-C and AER-C HRT ranges between 9.6-21 hrs, and 30-66.7 hrs, respectively. The corresponding average OLR's for ANX-C and AER-C ranges between 1.34-3.05 kg COD/m^3 d and 0.21-0.50 kg COD/m^3 d, respectively. Removal efficiencies of COD, BOD_5, TSS, nitrification efficiency, denitrification efficiency, TKN and TN ranges between 94.7-97.9 %, 97.8-99.0 %, 92.7-96.6 %, 94.7-96.7 %, 98.6-98.8 %, 96.6-97.7 %, and 80.6-82.8 %, respectively. The performance of bioreactor for organic matter and total nitrogen removal were achieved above 90 %, except for TN that was observed to be below 90 %. The reason for TN removal below 90 % was due to residual effluent nitrate concentration form AER-C. In phase III, ANX-C and AER-C HRT ranges between 6.4-9.6 hrs, and 20-30 hrs, respectively. The corresponding average organic loading rates for ANX-C and AER-C ranges between 1.77-2.65 kg COD/m^3 d and 0.49-0.79 kg COD/m^3 d, respectively. Removal efficiencies for COD, BOD_5, TSS, nitrification, denitrification, TKN and TN were obtained between the ranges of 95.2-97.9 %, 98.0-98.7 %, 91.2-94.6 %, 89.8-95.6 %, 98.8-98.9 %, 93.7-97.2 %, and 70.7-79.7 %, respectively. Variation of OLR in phase III was observed not to have significant influence on COD removal efficiency. The COD removal efficiency was observed in narrow range between 95.2-97.9 %. Increase in averages MLSS concentration to 5,139 mg/L in phase III could probably influence the increase in COD removal efficiency.

Denitrification in the anoxic chamber had nitrate removal efficiency continuously observed above 98 % irrespective of the HRT and OLR. Increase in ANX-C biomass concentration could be responsible to accomplish effective denitrification performance.

Conversely, the E-CLR nitrate concentration was observed to be higher with decreased HRT and increased OLR. Hence, it can be concluded that removal efficiency generally decreased with decrease in HRT. Similarly, higher loading rates applied to the bioreactor resulted in the reduction of system efficiency, which may be expected since any organic loading above the maximum microbial uptake will be untreated. Denitrification performance between IR ratio of 6 and IR ratio of 3 achieved average of 28.1 % higher denitrification efficiency. There was no effect on ammonia-nitrogen and COD concentration supplemental removal performance due to variation of IR ratio. The pre-denitrification and aerobic processes have accomplished effective reduction of total nitrogen and COD during the continuous operation of the system. Consequently, operation with i-SGBR system has conformed with national allowable limits for COD, BOD_5, ammonia-nitrogen and nitrate according to DoE Malaysia discharge standards.

Bio-kinetic coefficients were studied on sCOD basis to evaluate carbon oxidation for heterotrophic bacteria at average MLSS concentration of 5139 mg/L, and AER-C OLR between 0.49-0.79 kg COD/m^3 d. The values of the bio-kinetic coefficients were obtained as: Yield coefficient, Y = 0.72 mg VSS/mg COD, decay coefficient, K_d= 0.017d^{-1}, maximum specific growth rate, μ_{max} = 2.03 d^{-1} and half velocity constant, K_s = 979 mg sCOD/L, respectively. The experiment was performed at average temperature of 29.9±1.1 °C. The biokinetic coefficient evaluated would be useful to scale up operation and realize continuous system improvement.

Aerobic digestion was monitored with solids retention time of 10 days. Excess activated sludge from underflow was fed daily into the aerobic digester during phase II and phase III of the operation. Degradation of MLSS and MLVSS were monitored in the aerobic digester. pH in the AD was constantly monitored with observed average value of 7.1. Efficiency of aerobic digester was found to decline with increase of influent aerobic digester solids concentration. Odors, clogging or foaming in AD-C were not detected during operation of aerobic digester. Where in phase II, aerobic digestion performance achieved reduction between 14.4-19.3 % for MLSS concentration, and 20.4-31.7 % for MLVSS concentration. The range for IAD MLSS and MLVSS concentration were 6,299-12,515 and 4,798-10,719 mg/L, respectively. While, in phase III operation, aerobic digestion performance achieved MLSS reduction

between 7.3-14.3 % and MLVSS reduction between 9.8-18.6 %. Range for IAD MLSS and IAD MLVSS concentrations were 13,055-19,475 mg/L and 10,893-15,021 mg/L, respectively.

5.3 Research contributions

The following are main contributions of the research.

1. Development and integration of treatment units into a single bioreactor has offered a common wall construction approach to realize a compact wastewater treatment system. This compact system can be used as a packaged plant for easy mobility, deployment as well as accomplishing DoE Malaysia regulatory limits.

2. Treatment of beverage industry waterwater through integrated and compact wastewater system was successfully accomplished. Removal of COD, TSS and total nitrogen were satisfactory. Improvement over conventional methods was achieved, especially systems that are unable to attain reduction of nitrate nitrogen due to absence of anoxic chamber to perform denitrication functions.

3. Simultaneous operation of aerobic digester subsystem has reduced excess sludge production, with subsequent sludge handling in terms of wasting minimized.

5.4 Recommendation for future research

1. Modelling and data simulation for dynamic behavior of carbon oxidation, nitrification and denitrification may be considered for the prediction of process performance under various operational conditions and wastewater sources.

2. Integration of other devices with aerobic digester may enhance solubilization of COD to accomplish considerable degradation of sludge when higher sludge loading rate is operated (e.g sonication device, and alternating anoxic/aerobic cycles to monitor performance).

3. Nitrous oxide is among the intermediate pollutants in denitrification process. Wastewater treatment plant operations have been reported to contribute towards the green house emissions. Detailed analysis may be considered for nitrous oxide (N_2O) generation and mitigation of its effects.

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APPENDIX A

DESIGN WORK SHEET

Table A1.1: Design for BOD_5 removal and nitrification

	Design of Pilot Study Sewage Treatment Plant			**Extended Aeration Process**
	BOD Removal with Nitrification, Denitrification and Aerobic Digestion			
	Capacity: Flow Rate, Q	**150 L/d**	**L/day**	
Guidance: Yellow cells are input information to be suitably filled. Green cells are to be reviewed and modified/updated.				
S No	**Description of Parameter**	**Value**	**Unit**	**Reference**
	Quantity of Sewage Generated	**100.00**	**Lpd**	176
		0.10	**m^3/d**	
1	**Raw Sewage Characteristics Data**			
1	Average Sewage flow entering the treatment plant	100.00	lpd	
	Assume Peak Factor	1.50		
2	Peak Sewage flow entering the treatment plant, Q	0.150	**m^3/d**	
		150.00	**lpd**	
3	BOD	350	g/m^3	
4	sBOD	83	g/m^3	
5	COD	800	g/m^3	
6	sCOD	179	g/m^3	
7	rbCOD	113	g/m^3	
8	TSS	210	g/m^3	
9	VSS	146	g/m^3	
10	TKN	40	g/m^3	$mg/L = g/m^3$
11	NH_4-N	25	g/m^3	
12	Ne (Assumed effluent Ammonia concentration)	0.5	g/m^3	
13	TP	7	g/m^3	
14	Alkalinity	140	as $CaCO_3$	
15	bCOD/BOD ratio	1.6	g/m^3	
2	**Develop the Wastewater Characteristics needed for design**			
	a. bCOD			
	bCOD = 1.6(BOD)			
		560	**g/m^3**	
	b. nbCOD			
	nbCOD = COD - bCOD			
		240	**g/m^3**	

Table A1.1: Design for BOD_5 removal and nitrification cont.

S No	Description of Parameter	Value	Unit	Reference
	b. nbCOD			
	nbCOD = COD - bCOD			
		240	g/m^3	
	c. sCOD effluent			
	$sCOD_e$ = sCOD - 1.6sBOD			
		46.99	g/m^3	
	d. nbVSS			
	bpCOD/pCOD = (1.6(BOD-sBOD)/(COD-sCOD))			
		0.69		
	nbVSS = (1 - bpCOD/pCOD)VSS			
		45.34	g/m^3	
	e. iTSS			
	iTSS = TSS - VSS			
		64.17	g/m^3	
2	Design for suspended growth for BOD removal and Nitrification			
$P_{x,bio}$	$=(QY(So-S))/(1+(k_d SRT) + ((fd)(kd)\ Q(Y)(So-S)SRT)/(1+(k_d SRT) + (QYn(NOx))/(1+(k_{dn} SRT)$ ..... (1)			
a				
3	Determine the specific growth rate for the denitrifying organisms			
	Note that it is essential to calculate the specific growth rate for the denitrifying organism because they grow more slowly than the heterotrophic organism that remove organic carbon. This means that they control the tank design. (Eq 7-93)			
	$\mu_{a,m} N$ DO			
	μ_a = (----------) (----------) - kdn ..... (2)			
	$K_a + N$ $K_a + DO$			

Table A1.1: Design for BOD_5 removal and nitrification cont.

	Design of Pilot Study Sewage Treatment Plant			Extended Aeration Process
	BOD Removal with Nitrification, Denitrification and Aerobic Digestion			
	Capacity: Flow Rate, Q	150 L/d	L/day	
Guidance: Yellow cells are input information to be suitably filled. Green cells are to be reviewed and modified/updated.				
S No	Description of Parameter	Value	Unit	Reference
	$\mu_n = \mu_{n,m}\left(\frac{N}{K_n+N}\right)\left(\frac{DO}{K_o+DO}\right) - k_{dn}$ ..... (2)			
	Where:			
	μ_n = specific growth rate of nitrifying bacteria, g new cells/g cells.d			
	$\mu_{n,m}$ = maximum specific growth rate of nitrifying bacteria, g new cell/g cell.day			
	N = nitrogen concentration, g/m^3			
	K_n = half-velocity constant, substrate concentration at one-half the maximum specific substrate utilization rate, g/m^3			
	K_o = half-saturation coefficient for DO, g/m^3			
	kd = endogenous decay coefficient, g VSS/g VSS.d			
	DO = dissolved oxygen concentration, g/m^3			
	k_{dn} = endogenous decay coefficient for nitrifying orgaism, g VSS/g VSS.d			
4	Kinetic Coefficients, DO, MLSS and Temperature for Design			
				Kinetic coefficients adopted from Metcalf & Eddy, 4th Ed.
	Y	0.4	gVSS/gbCOD	
	So	560	bCOD/m^3	
	Ks	20	g/m^3	
	SRT	40	Days	
	fd	0.15		
	X_{TSS}	5000	g/m^3	
	N	0.5	g/m^3	
	DO	2	g/m^3	
	Ko	0.5	g/m^3	
	Yn	0.12	gVSS/gNH_4-N	
	T	28	°C	
	Determine the specific growth rate, μn from Step 3 and 4			

Table A1.1: Design for BOD_5 removal and nitrification cont.

S No	Description of Parameter	Value	Unit	Reference
5	Determine the theoretical and design SRT			
	(a) Find theoretical SRT			Metcalf & Eddy 4th Ed., (Eq 7-37)
	$SRT = \left(\frac{1}{\mu_n}\right)$ .....(3)	4.79	days	
	(b) Find design SRT (Eq 7-71)			
	FS = TKN peak / TKN average =	1.5		
	Design SRT = (FS) (Theoretical SRT)			
	= 1.5 * 4.79	7.18		
	Assume Design SRT to be according to window for extended aeration ASP	20.00	days	Metcalf & Eddy, 4th Ed., Table 8-16, pg. 746
6	Determine Biomass production			
	a. Determine S first and substitute in $P_{x,bio}$			
	$S = \left(\frac{K_s [1 + (kd) SRT]}{SRT (\mu m - kd) - 1}\right)$ ..... (4)			S is determined from Eq 7-40 in Table 5
	Use the following:			
	K_s	20.00	g/m^3	Table 8-10
	μ_m	6	g/g.d	
	$\mu_{m,T}$			

Table A1.1: Design for BOD_5 removal and nitrification cont.

S No	Description of Parameter	Value	Unit	Reference
	= C135*(1.07^(C87-20)) = $\mu_m \Theta^{(T-20)}$	10.31	g/g.d	
	Hence S = (D128*(1+(C113*C97)))/((C97*(D130-C113)-1))	0.42	g bCOD/m^3	
	b. Assume NO_X is 80% of TKN as nitrogen balance cannot be done yet. The error in assuming that the NOX is 80%(TKN) is small as nitrifier VSS yield is a small fraction of total MLVSS concentration.			
	NO_X = 0.85 * TKN	34.00	g/m^3	
	c. To resolve $P_{x,bio}$			
	$(P_{x,bio}=(QY(So-S))/(1+(k_d)SRT) + ((fd)(kd)\ Q\ (Y)(So-S)SRT)/(1+(k_d)SRT) + (QYn\ (NOx))/(1+(k_{dn})SRT)$ ..... (1			
				-13.37499922
	= Part A + Part B + Part C			
	= Hetetrophic Bacteria biomass + Cell debris + Nitrifying bacteria biomass			
	PART A	0.007836	kg VSS/d	
	PART B	0.003861	kg VSS/d	
	PART C	0.000192	kg VSS/d	
	Px, bio	0.011889	kg VSS/d	
		11.889	g VSS/d	
7	Determine the amount of Nitrogen oxidized to nitrate			
				The amount of nitrogen oxidized to nitrate can be found by performing nitrogen balance using Eq. (8-18)
	$NO_X = TKN_{inf} - Ne - 0.12\ P_{x,bio} / Q$ (5)	30.0	g/m^3	
				(If (-) value is obtain, consider nitrogen is completely oxidized)

Table A1.1: Design for BOD_5 removal and nitrification cont.

S No	Description of Parameter	Value	Unit	Reference
				(If (-) value is obtain, consider nitrogen is completely
8	Detemine the concentration and mass of VSS and TSS in aeration basin			
	Mass = P_X (SRT)			
	(a) Calculate the concentration of VSS and TSS in aeration basin			
	(i) $P_{X,VSS} = P_{x,bio} + Q$ (nbVSS) (6)			Use Eq 8-15, parts A, B and C have already been determined as $P_{x,bio}$, therefore part D must be added to get $P_{x,VSS}$
	$P_{x,bio}$	0.0119	kg/d	
	Q (nbVSS)	0.0068	kg/d	
	$P_{x,vss}$	0.0187	kg/d	
	(ii) $P_{X,TSS} = (P_{x,bio} / 0.85) - Q$ (nbVSS) + Q(TSS_o - VSS_o) (7)			
				Use Eq 8-16 with the term E added to account for inert influent TSS
	(Px,bio / 0.85)	0.0140	kg/d	
	Q (nbVSS)	0.0068	kg/d	
	Q(TSSo - VSSo)	0.0096	kg/d	
	$P_{x,vss}$	0.0304	kg/d	
	(b). Calculate the mass of VSS and TSS in the aeration basin			*Use Equation 7-54 and 7-55 in Table 8-5 Metcalf & Eddy, 4th Ed*
	(i) Mass of MLVSS			
	$(X_{VSS})(V) = (P_{x,VSS})$ SRT (8)			
	$(P_{x,VSS})$	0.0187	kg/d	
	SRT	20.00	days	
	$(X_{VSS})(V) = (P_{x,VSS})$ SRT	0.3738	kg	

Table A1.1: Design for BOD_5 removal and nitrification cont.

S No	Description of Parameter	Value	Unit	Reference
	(X_{TSS}) $(V) = (P_{x,TSS})$ SRT	*0.3738*	kg	
	(ii) Mass of MLSS			
	(X_{TSS}) $(V) = (P_{x,TSS})$ SRT (9)			
	$(P_{x,TSS})$	*0.0304*	kg/d	
	SRT	*20.00*	days	
	(X_{TSS}) $(V) = (P_{x,TSS})$ SRT	*0.6082*	kg	
9	**Select a design MLSS mass concentration and determine the aeration tank volume and detention time using the TSS mass computed in previous step**			
	a. Determine the aeration tank volume			
	(V) (X_{TSS}) = 0.8374 kg			
	At MLSS = 5000 g/m^3			
	Volume, V =	**0.12**	**m^3**	
		121.65	**liters**	
	b. Determine aeration tank detention time			
				64.88
	$\tau = \frac{V}{Q}$ .....Equation 10	**19.5**	**hrs**	
	c. Determine MLVSS			
	Fraction VSS = Mass of MLVSS / Mass of MLSS	*0.61*	Unitless	
	Hence, MLVSS = 0.53 * (5000 g/m^3)	*3073*	g/m^3	

Table A1.1: Design for BOD_5 removal and nitrification cont.

S No	Description of Parameter	Value	Unit	Reference
	Hence, MLVSS = 0.53 * (5000 g/m³)	3075	g/m³	
10	Determine F/M and BOD Volumetric loading			
	a. Determine			
	F QSo g BOD	0.086	g/g.d	(Range: 0.04 - 0.1) g/g.d
	..... = ... = ...			Met&Eddy, p. 747
	M XV g MLVSS.d 11			
				Using Equation (7-51)
	b. Determine volumetric BOD loading			
	QSo kg BOD	0.691	kg/m³.d	
	L_{org} = ... = ...			
	V m³.d 12			
11	Determine the observed yield based on TSS and VSS			
	Observed yield = g TSS / g bCOD = kg TSS / kg bCOD 13			
	$P_{X,TSS}$ =	0.0304	kg/d	
	bCOD removed = Q (So - S)	0.0540	kg/day	
	(a) Observed yield based on TSS			
	$Y_{obs,TSS}$ = (2032.4 kg/d) / (5009.5 kg/d) = (0.41 kg TSS/kg bCOD)	0.56	g TSS/g bCOD	
		0.58	g TSS/g BOD	
	(b) Observed yield based on VSS			
	$Y_{obs,VSS}$ / VSS/TSS = 0.53	0.0187	g VSS/g bCOD	
		0.0299	g VSS/g BOD	

12	Calculate the O_2 demand			
	R_O = Q (So - S) - 1.42 $P_{x,bio}$ + 4.33Q (NO_3) 14			(Eq 8-17)
	Q (So - S)	0.0540		
	1.42 $P_{x,bio}$	0.0169		
	4.33Q (NO_3)	0.0195		
	R_O = Q (So - S) - 1.42 $P_{x,bio}$ + 4.33Q (NO_3)	**0.1204**	**kg/d**	
		0.0050	**kg/h**	

Table A1.1: Design for BOD_5 removal and nitrification cont.

S No	Description of Parameter	Value	Unit	Reference
13	Check Alkalinity in Aerobic system			
	(a) Prepare an alkalinity balance			
	Alkalinity to maintain pH - 7 = Influent Alk - Alk used + Alk			
	Influent Alkalinity :	140	g/m^3 as $CaCO_3$	
	Amount of nitrogen to be converted to nitrate, NO_N =	30.0	g/m^3	
	Alkalinity used for nitrification	214.1	g/m^3 used as $CaCO_3$	
	(b) Substitude known values and solve for alkalinity needed			
	Residual alkalinity concentration needed to maintain pH in range 6.8-7.0 is 70 to 80 g/m^3 as $CaCO_3$	80	g/m^3	
	Hence, Alkalinity to be Added =	154.1	g/m^3	(Note that if (+), alkalinity need to be added)
		0.023	kg/d as $CaCO_3$	
		23.12	g/d	
	(c) Determine the alkalinity needed as sodium bicarbonate			
	Equivalent weight of $CaCO_3$ = 50g / equivalent	50		
	Equivalent weight of $Na(HCO_3)$ = 84g / equivalent	84		
	Hence, Na(HCO3) needed =	38.64	g/d $NaHCO_3$	
14	Estimate effluent BOD			
	Assume			*Metcalf & Eddy, 4th Ed. (Eq 8-25)*
	sBODe =	3.0	g/m^3	
	TSS =	10.0	g/m^3	
	$BOD_e = sBOD_e + (\frac{g\,BOD}{1.42\,g\,VSS} \times \frac{0.85\,g\,VSS}{g\,TSS}) \times (TSS, g/m^3) \times \frac{1}{15}$			
		8.99	g/m^3	
	Efficiency =	97.4	%	

Table A1.1: Design of clarifier

S No	Description of Parameter	Value	Unit	Reference
15	Secondary Clarifier design for both BOD removal and Nitrification			
	(a) Define return sludge recycle ratio			Figure 8-9 Metcalf & Eddy, 4th Ed., Mass balance around sec. clarifier
	$Q_r X_r = (Q + Q_r) X$ (assume water sludge mass is insignificant) Eq 16			
	Q_r = RAS flowrate		m^3/day	
	X_r = return sludge mass concentration		g/m^3	
	R = RAS recycle ratio = Q_r / Q			
	Hence R = $\frac{X}{X_r - X}$	17		
	(b) Determine the size of clarifier			
	Assume, X_r = 15000 g/m^3	15000	g/m^3	
	Hence R =	0.500		
	Assume a hydraulic application rate (HAR) of 22 m^3/m^2.d at average flow for the secondary clarifier	**24**	**m^3/m^2.d**	Table 8-7, range is 16 to 28 m^3/m^2.d, page 687
	Area = Peak flow, Q / HAR =	0.006250	m^2	
		6250.00	cm^2	
	Use 1 No. Clarifier unit			
	Clarifier radius =	0.04	m	
	Clarifier Diameter =	0.09	m	
		9.92	cm	
	Use diameter 1.5ce as one calculated to comply with range for solids loading of 4-6 kg/m^2.d	0.13	m	

	(c) Check solids loading			
	Solids Loading = $\frac{(1+R)\,Q\,[MLSS]}{A} = \frac{(1+R)\,Q\,(MLSS)}{A}$ Equation 18			
	where A = area of clarifier, m^2 = (3.142) (0.05m)^2 / 4 =	0.0063	m^2	
		6250.00	cm^2	
	Solids loading =	3.33	kg MLSS/m^2.h	Not consistent with solid loading of range between 1.0 - 6 (peak 7) kg/m^2.d, Table 8-7
	Use **diameter** 1.5ce as one calculated to comply with range for solids loading of 7 kg/m^2.d (Settling following extended aeration)	0.13	m	
	New Area of clarifier =	0.0141	m^2	
	New Solids loading =	3.33	kg MLSS/m^2.h	Acceptable, as not exceeding 7 kg MLSS/m^2.h according to table 8-7, page 687

Table A1.2: Design for denitrification cont.

S No	Description of Parameter	Value	Unit	Reference
	From bottom of slope to effluent channel, use h = 0.75 m =	0.80	m	
	Volume =	0.10	m^3	100.54
	Allow free board of 10 cm =	0.10	m	
	Total height of clarifier tank =	0.90	m	
16	Dimensioning Anoxic tank			
	Calculated volume of Anoxic tank =	0.05	m^3	
	Use 250 mm Diameter steel cylinder of 3mm thick			
	Area of anoxic to be implemented = (3.142 * ((250)/1000)^2)/4 =	0.05	m^2	
	Try h= 0.41 m	0.90	m	
	Volume of the anoxic tank =	0.04	m^3	
16	Dimensioning Digester			
	Calculated volume of Digester tank =	0.07	m^3	
	Use 800 mm Diameter steel cylinder of 3mm thick			
	Area of Digester to be implemented = (3.142 * ((800-650)/1000)^2) =	0.13	m^2	
	adopt h= 0.80m	0.80	m	
	New Volume of digester to be implemented =	0.10	m^3	
	Allow free board of 150mm from end side digester =	0.15	m	
	Total height =	0.95	m	

Table A1.2: Design for denitrification cont.

S No	Description of Parameter	Value	Unit	Reference
	Quantity of Sewage Generated	100.00	Lpd	
		0.10	m^3/d	
1	Wastewater Characteristics Data			
1	Average Sewage flow entering the treatment plant	100.00	lpd	
	Assume Peak Factor	1.50		
2	Peak Sewage flow entering the treatment plant, Q	0.150	m^3/d	
		150.00	lpd	
3	BOD	600	g/m^3	
6	bCOD	560	g/m^3	
7	rbCOD	350	g/m^3	Note:
10	NOx	29.99	g/m^3	mg/L = g/m^3
12	Ne (Assumed NO_3-N concentration in RAS)	4	g/m^3	
13	TP	22	g/m^3	
14	Alkalinity	440	as $CaCO_3$	
15	Residual Alkalinity	80.00	as $CaCO_3$	
2	Design conditions:			
	Paramenter			Assumptions:
	Influent flowrate	0.15	m^3/day	1. Nitrate concentration in RAS = 6 g/m^3
	Temperature	28	°C	2. Use the same coefficients as the nitrification process design
	MLSS	5000	g/m^3	3. Mixing energy for anoxic reactor = 10 $kW/10^3 m^3$.
	MLVSS	3073	g/m^3	
	Aerobic SRT	20.00	d	
	Aeration basin volume	0.12	m^3	
	Mixing energy	10	$kW/10^3 m^3$	
	RAS ratio	0.60	Unitless	
	R_r	0.0050	kg/h	

Table A1.2: Design for denitrification cont.

S No	Description of Parameter	Value	Unit	Reference
3	Determine the active biomass concentration			
	Note: Subtitude V/Q for τ			(Eq 7-43), substitute for v/Q for τ
	$X_o = \left[\frac{Q(SRT)}{V}\right]\left[\frac{Y(So-S)}{1+(kd)SRT}\right]$ Equation 11			
	where So - S can be assume as So			
	Y =	0.4	VSS/g bCOD	
	So =	560	g bCOD/m^3	
	S =	0.42	g bCOD/m^3	
	k_d =	0.16	g/g.d	
	X_b =	1,289.29	g/m^3	
4	Determine the amount of nitrate produced in Digester			
	Overall equation with complete nitrification			pg. 1534 Aerobic digestion
	$C_5H_7NO_2 + 7O_2 \longrightarrow 5CO_2 + 3H_2O + HNO_3$			
	$HNO_3 \longrightarrow H^+ + NO_3^-$			
	113mg of biomass produces 62 mg nitrate			
	Daily RAS flow to digester is 7.5 Liter	7.5	lpd	
	Therefore, 113mg/L biomass produces 62 mg/L nitrate			
	1 mg/L biomass produces 0.5486 mg/L nitrate in digester		lpd	
	Flow to the digester =	7.5	m^3/d	
	Assume 15,000 mg/L MLSS in RAS	15000	g/m^3	
	MLVSS concencentration = 15000 * 0.85 mg/L	12750	g/m^3	
	Nitrate produced in the digester = (X_{VSS} mg/L) * (62 mg / 113 mg)) =	6,995.58		*62m is atomic weight of Nitrate (16*3 * 14) = 62 mg
				*113 mg is atomic weight of biomass
	Mass of Nitrate in Aeration tank (125 L) from Digester	55.96	mg	
	Concentration of nitrate from Digester in Aeration tank , Digester flow = 7.5 lpd	7.46	mg/L	

Table A1.2: Design for denitrification cont.

S No	Description of Parameter	Value	Unit	Reference
5	Determine the IR ratio due to Aerobic tank + Digester			
				(Eq 8-48).
	Aerobic tank NO_3-N effluent concentration = Ne =	4.00	g/m^3	
	NO_x			
	IR = ------- - 1.0 - R Equation 12			
	Ne			
	= (C18 + C70 g/m^3) / (4 g/m^3) -1.0 - C32	8	unitless	
6	Determine the amount of NO_3-N fed to the anoxic tank from Aeration tank			
	Flow rate to anoxic tank = IR Q + R Q=	1.25	m^3/d	
		1254.41	lpd	
	NO_x feed = (1.57 m^3/d) (4.0 g/m^3) =	5.02	g/d	
7	Determine the anoxic volume due to Aerobic + Digester			
	As a first approximation, use a detention time = 8 h	8.0	h	
	Vnox = T * Q Equation 13			
	Detention time = 8 h / (24 h/d) =	0.33	day	
	Vnox =	0.05	m^3	
		52.50	liters	
	Provide anoxic to nearest 10th Liter for Denitrification		liters	

Table A1.2: Design for denitrification cont.

S No	Description of Parameter	Value	Unit	Reference
	Provide anoxic to nearest 10th Liter for Denitrification		liters	
8	Determine Food to microorganisms ratio (F/M_b)			
				Using Equation (8-43)
	$\frac{F}{M_b} = \frac{QS_o}{V_{nox}(X_a)}$ Equation 14			Terms previously defined
	Hence F/M_b =	1.33	g/g.d	
9	Determine the SNDR using the curve with an F/M_b range of 0 to 4			
				Figure 8-23, page 755
	Fraction rbCOD = rbCOD/bCOD = (350 g/m^3) (960 g/m^3)	0.63	%	
	From Figure 8-23, $SDNR_0$ = 0.35 g/g.d at 20°C	0.35	g/g.d	
	$SNDR_{23} = 0.35\ (1.026)^{(23-20)}$ =	0.43	g/g.d	
10	Determine the amount of NO_3-N that can be reduced			
				Equation (8-41)
	a. Check NOr based on detention time = 8 h	8.00	h	
	NOr = (Vnox) (SDNR) (MLVSS, biomass)	29.09	g/d	Compare with value ref. C69
	Comparing NOr = 3.51 and NOx = 4.78 g/d			
	If it exceeds then the lower detention time may be used and vice versa			

11	Determine the Oxygen saving and net oxygen for Nitrif.			
	R_o (without denitrification) =	0.0050	kg/h	Refer to step 12, BOD Rem. + Nitrificatn
	Oxygen credit = (2.86 O_2/ g NO_3-N) [112.1 - 6.0] g/m^3 (0.045 m^3/d) (1kg/10^3 g)			
	=	0.0111	kg/d	
		0.000464553	kg/h	
	Net Oxygen required =	0.0046	kg/h	
				9.26
	Note the required aeration rate will decrease in proposrtion to a lower Ro. The oxygen required can be reduced by 20 percent.			

Table A1.2: Design for denitrification cont.

S No	Description of Parameter	Value	Unit	Reference
12	Check alkalinity			
	(a) Prepare an alkalinity balance			
	Alkalinity to maintain pH - 7 = Influent Alk - Alk used + Alk to be added			
	i. Influent alkalinity	440.00	g/m^3	
	ii. Alkalinity used	214.12	g/m^3	
	iii. Alkalinity produced	92.78	g/m^3	
	iv. Alkalinity needed to maintain neutral pH	80.00	g/m^3	as $CaCO_3$
	(b) Solve the above equation for alkalinity to be added			
	Alk to be added = (iv - i+ ii- iii) above =	-238.66	g/m^3	as CaCO3
	Mass of alkalinity needed =	-0.0358	kg/d	
		-35.7989	g/d	
	(c) Compare to alkalinity needed for nitrification			
	For the nitrification only design, the alkalinity needed was	0.0231	kg/d	
	Alkalinity savings =	0.0589	kg/d	254.9
13	Determine anoxic zone mixing energy			
	Mixing energy = 10 $kW/10^3$ m^3 (given)	0.0100		
	Anoxic volume due to Aeration =	**0.0525**	m^3	
	Power = (Anoxic volume,m^3) * (10 $kW/10^3$ m^3) =	0.000525	kW	
		0.5250	W	
	Use 0.5 W submersible pump with speed rpm (speed) controller	0.5000	W	

Table A1.2: Design for aerobic digestion

S No	Description of Parameter	Value	Unit	Reference
1	Wastewater Characteristics Data			
1	Average Sewage flow entering the treatment plant	30.00	lpd	
	Assume Peak Factor	1.50		
2	Peak Sewage flow entering the treatment plant, Q	0.045	m^3/d	
		45.00	lpd	
3	BOD	600	g/m^3	
6	bCOD	560	g/m^3	
7	rbCOD	350	g/m^3	Note:
10	NOx	29.99	g/m^3	$mg/L = g/m^3$
12	Ne (Assumed NO_3-N concentration in RAS)	6	g/m^3	
13	TP	22	g/m^3	
14	Alkalinity	440	as $CaCO_3$	
15	Residual Alkalinity	80.00	as $CaCO_4$	
2	Design conditions:			
	Paramenter			Assumptions:
	Influent flowrate	7.5	m^3/day	1. Nitrate concentration in RAS = 6 g/m^3
	Temperature	28	°C	
	MLSS	10000	g/m^3	
	MLVSS	3073	g/m^3	
	Aerobic SRT	10.00	d	

Table A1.2: Design for aerobic digestion cont.

3	Design the Endogenous Sludge Digestion in the Digester			
	The digester tank volume can be calculated by equation 14-22 (WEF, 1988), Metcalf & Eddy			
	$V=(Qi(Xi-YSi))/(X(kdPv+1/SRT)$	**Equation 14**		Mackenzie L. Davies, Section 27-33
				Metcalf & Eddy 4th Ed., pg. 1540
	Where V = Volume of aerobic digester, m^3 (ft^3)			
	Qi = influent average flow rate to digester, m^3/d = volume to be disposed of per day	7.5	lpd	
		0.0075	m^3/d	
	Xi = influent suspended solids, mg/L = concentration of RAS from clarifier	10000	g/m^3	
	Y = fraction of the influent BOD consisting of raw primary solids, (expressed as a decimal)			
	X = digester suspended solids, mg/L			
	kd = reaction rate constant, d^{-1}	$0.06d^{-1}$		
	Pv = volatile fraction of digester suspended solids (expressed as decimal)			
	SRT = solids retention time, d	10	days	
	Note: The term YSi can be neglected if primary sludge is not included in the sludge load to the aerobic digester.			
	Assume volatile fraction of digester TSS is 0.80			
	Sludge conc. In digester is 70% of the incoming thickened sludge conc.			
	Assume air temperature in diffused air system = 20°C			
	Compute Volatile solids reduction at 28 Degree centigrade			
	From Figure 14-31, assuming MCRT of 40 days			
	Degree-days = 28 X 10 = 280	280	degree-days	(Page 1540)

27-40

Tank Volume. The volume of the digester tank may be estimated with the following equation (WEF, 1998):

$$V = \frac{Q(Y_o L_o + P_{bs})}{X(k_d P_v + 1/SRT)} \qquad (27\text{-}44)$$

where V = volume of aerobic digester, m^3
Q = average flowrate to digester, m^3/d
L_o = influent suspended solids, mg/L
P_{bs} = fraction of influent BOD that is raw primary solids
S = digester influent BOD, mg/L
X = digester suspended solids, mg/L
k_d = reaction rate constant, d^{-1}
P_v = volatile fraction of digester suspended solids
SRT = solids retention time, d

The term P_{bs} can be ignored if primary sludge is not included in the sludge feed to the digester. Representative values for k_d range linearly from 0.03 d^{-1} at 10°C to 0.14 d^{-1} at 25°C for waste activated sludge (U.S. EPA, 1979). However, site or pilot-scale studies are recommended to obtain site-specific decay coefficients.

Table A1.2: Design for aerobic digestion cont.

3	Compute Volatile solids reduction at 28°C			
	Volatile solids reduction at 28°C will be 35%	0.0118888	kg/d	
4	Determine oxygen requirement			
				(see Table 14-34 for oxygen requirement)
	Oxygen requirement =	0.0273442	kg O_2/d	
		27.34	g O_2/d	
		0.001139	kg/h	
5	Volume of sludge to be wasted daily based on RAS line			
	Qw = (V.X) / (SRT. Xr) Equation 16			
	V = vol. of aeration tank	0.1216496	m^3/d	
	X = MLSS conc. In aeration tank	5000	g/m^3	
	Xr = RAS conc.			
	Qw= (C83 m^3 * 5000 g/m^3) / (40 days * 15000g/m^3)	0.0060825	m^3/d	
		6.0824785	lpd	
	Assume Design digester inflow	7.5	lpd	
		0.0075	m^3/d	
6	Volume of Digester			
	Volume of Digester =	0.0723938	m^3	
		72.393822	liters	

TABLE 27-12
Typical design criteria for aerobic digesters

Parameter	Range of values
Feed concentration	1.5–3.5%
SRT to meet PSRP[a]	
At 15°C	60 d
At 20°C	40 d
k_d at 10°C	0.02 d^{-1}
k_d at 15°C	0.06 d^{-1}
k_d at 20°C	0.10 d^{-1}
k_d at 25°C	0.14 d^{-1}
Volatile solids loading	1.6–4.8 kg/m^3 · d
Oxygen requirements:	
Cell tissue	~2.3 kg O_2/kg VSS destroyed
BOD_L in primary sludge	1.6–1.9 kg O_2/kg VSS destroyed
Oxygen concentration	≥ 1 mg/L
Air flow rates for oxygen	
Waste activated sludge (WAS)	0.015–0.020 m^3/min · m^3
Mixed primary and WAS	0.024–0.030 m^3/min · m^3
Mixing requirements	
Mechanical aerators/mixers	20–40 kW/10^3 m^3
Diffused air mixing	0.02–0.040 m^3/min · m^3
Reduction in VSS	38–50%
Tank dimensions	
Depth for diffused air	4.5–7.5 m
Depth for mechanical air	3–6 m
Circular diameter[b]	12–45 m
Rectangular	
W:D	1:1 to 2.2:1
L:W	≥ 5:1

[a]PSRP = process to significantly reduce pathogens.
[b]Circular is the typical configuration.
Sources: 40 CFR 503; Metcalf & Eddy, 2003; U.S. EPA, 1979; WEF, 1998.

Table A1.3: Design for aerobic digestion cont.

6 Volume of Digester			
Volume of Digester =	0.0723938	m^3	
	72.393822	liters	
Use digester volume of 75 liters for the aerobic digester			
7 Check Alkalinity in Aerobic system			
(a) Prepare an alkalinity balance			
Alkalinity to maintain pH - 7 = Influent Alk - Alk used + Alk to be added			
Influent Alkalinity:	440	g/m^3	
Amount of nitrogen to be converted to nitrate, NO_x =	6996	g/m^3	
Alkalinity used for nitrification	49948	g/m^3	
(b) Substitude known values and solve for alkalinity needed			
Residual alkalinity concentration needed to maintain pH in range 6.8-7.0 is 70 to 80 g/m^3 as $CaCO_3$			$\mu = \frac{270\ m^3/d[38,000\ g/m^3 + (0.35)(5,200\ g/m^3)]}{(38,000\ g/m^3)(0.7)(0.06\ d^{-1})(0.80) + 1)/60\ d]}$
Hence, Alkalinity to be Added =	56504	g/m^3 as $CaCO_3$	$= \frac{1.08 \times 10^3}{1.72 \times 10^5} = 6.25 \times 10^{-3}\ m^3$
	0.4237799	kg/d	
	423.77987	g/d	

Circular diameter[b]	12-45 m
Rectangular	
W:D	1:1 to 2.2:1
L:W	≥ 5:1

[a]PSRP = process to significantly reduce pathogens.
[b]Circular is the typical configuration.
Sources: 40 CFR 503; Metcalf & Eddy, 2003; U.S. EPA, 1979; WEF, 1998.

TABLE 27-11
Characteristics of supernatant from aerobic digestion systems

Parameter	Range, mg/L	Typical, mg/L
BOD_5	9-1,700	500
Filtered BOD_5	4-183	50
COD	288-8,140	2,600
Kjeldahl nitrogen	10-400	170
Nitrate-N	N/A	30
Total P	10-241	100
Soluble P	2.5-64	25
Suspended solids	46-11,500	3,400

Adapted from WEF, 1998.

Table A1.3: Design summary output

Design Parameter	Unit	Value
Average wastewater flow	m^3/d	0.1500
Average BOD Load	kg/d	350
Average TKN load	kg/d	40
Aerobic SRT	d	40
Aeration tanks	Number	1
Aeration tank volume, ea	m^3	0.1216
Hydraulic detention time, T	h	19
MLSS	g/m^3	5000
MLVSS	g/m^3	3073
F/M	g/g.d	0.0863
BOD loading	kg BOD/m^3.d	
Sludge production	kg/d	0.0119
Observed yield	kg TSS/kg .bCOD	0.36
	kg VSS/kg .bBOD	
Oxygen required	kg/h	0.0050
RAS ratio	Unitless	0.50
Clarifier hydraulic application rate	$m^3/m^2.d$	24
Clarifiers	Number	1
	Diameter, m	
Alkalinity addition as CaCO3	kg/d	0.0231
Effluent BOD	g/m^3	8.99
TSSe	g/m^3	< 15
Effluent NH_4 - N	g/m^3	< 0.5

ANOXIC TANK FOR DENITRIFICATION

Design Parameter	Unit	Value
Effluent NO_3-N	g/m^3	4
Internal recycle ratio	Unitless	6
RAS recycle ratio	Unitless	1.00
Anoxic volume	m^3	0.05
MLSS	g/m^3	5000
Overall SDNR	g NO_3-N/g MLSS.d	0.43
Detention time	h	8
Mixing power	kW	0.5
Alkalinity required	kg/d as $CaCO_3$	-0.0358

DIGESTER FOR AEROBIC SLUDGE DIGESTION

Design Parameter	Unit	Value
Effluent NO_3-N	g/m^3	4,663.72
Flow to digester	m^3/d	0.0075
Digester volume	m^3	0.072393822
MLSS in RAS	g/m^3	10000
Alkalinity required	kg/d as $CaCO_3$	0.281419912

APPENDIX B

DATA FOR OPERATION AND CONTROL PARAMETERS

Table B1.1: Phase II: Sludge data ANX-C, AER-C & RAS MLSS AND MLVSS

DATE	DAY	ANOXIC MLSS, (mg/L)	ANOXIC MLVSS, (mg/L)	ANOXIC MLVSS/MLSS RATIO	AVERAGE MLSS AERATION (mg/L)	AVERAGE MLVSS AERATION (mg/L)	SRT, (d)	Settled sludge volume, SSV (mL/g)	Sludge Volume Index (SVI), mL/g = (SSV*1000 / MLSS)	AERATION MLVSS / MLSS RATIO	RAS OBSERVED, (mg/L)	RAS PREDICTED, Xr = 10^6/SVI, (mg/L)(mg/L)
08/06/15	1	1642	1525	0.9	1723	1440	38	920	534	0.8		1,873
10/06/15	3	1683	1396	0.8	1760	1393	37	897	510	0.8		1,962
12/06/15	5	1722	1325	0.8	1813	1410	41	853	470	0.8	2,107	2,126
15/06/15	8	1764	1214	0.7	1860	1333	39	825	444	0.7	2,310	2,255
17/06/15	10	1803	1293	0.7	1897	1363	37	700	369	0.7	-	2,710
19/06/15	12	2065	1418	0.7	2067	1413	41	655	317	0.7	3,077	3,155
22/06/15	15	2203	1500	0.7	2283	1590	41	679	297	0.7	3,130	3,363
24/06/15	17	2447	1600	0.7	2440	1710	39	665	273	0.7	-	3,669
26/06/15	19	2744	1924	0.7	2443	1740	37	609	249	0.7	3,873	4,012
29/06/15	22	2676	2006	0.7	2530	1847	35	569	225	0.7	4,233	4,446
01/07/15	24	2850	2347	0.8	2620	2100	38	582	222	0.8	-	4,502
03/07/15	26	3026	2526	0.8	2885	2350	49	541	188	0.8	4,880	5,333
06/07/15	29	3156	2493	0.8	3020	2603	45	439	165	0.9	5,593	6,052
08/07/15	31	3363	2556	0.8	3177	2693	44	529	167	0.8	-	6,005
10/07/15	33	3499	2712	0.8	3063	2633	41	499	163	0.9	6,260	6,139
13/07/15	36	3153	2517	0.8	3230	2843	43	507	157	0.9	5,920	6,371
15/07/15	38	3472	2792	0.8	3077	2680	39	466	151	0.9	-	6,602
17/07/15	40	3220	2417	0.8	3140	2710	41	495	158	0.9	6,017	6,343
20/07/15	43	3501	2657	0.8	3053	2640	41	465	152	0.9	6,250	6,566
22/07/15	45	3447	2563	0.7	3280	2867	41	472	160	0.9	-	6,250
24/07/15	47	3475	2678	0.8	2963	2570	41	494	167	0.9	5,990	5,999
27/07/15	50	3376	2656	0.8	3100	2747	38	454	146	0.9	6,867	6,828
29/07/15	52	3507	3083	0.9	3010	2560	40	466	155	0.9	6,323	6,459
31/07/15	54	3697	3227	0.9	3580	3167	42	498	139	0.9	6,873	7,189

Table B1.1: Phase II: Sludge data ANX-C, AER-C & RAS MLSS AND MLVSS Cont.

DATE	DAY	ANOXIC MLSS, (mg/L)	ANOXIC MLVSS, (mg/L)	ANOXIC MLVSS/MLSS RATIO	AVERAGE MLSS AERATION (mg/L)	AVERAGE MLVSS AERATION (mg/L)	SRT, (d)	Settled sludge volume, SSV (mL/g)	Sludge Volume Index (SVI), mL/g = (SSV*1000 / MLSS)	AERATION MLVSS / MLSS RATIO	RAS OBSERVED, (mg/L)	RAS PREDICTED, Xr = 10^6/SVI, (mg/L)(mg/L)
03/08/15	57	3547	2977	0.8	3273	2893	40	486	148	0.9	6,330	6,735
05/08/15	59	3412	2822	0.8	3373	3000	41	528	157	0.9	5,877	6,389
07/08/15	61	3504	3031	0.9	3030	2627	36	463	153	0.9	6,123	6,544
10/08/15	64	3530	2946	0.8	3163	2753	43	452	143	0.9	5,780	6,999
12/08/15	66	3448	2944	0.9	2923	2510	38	459	157	0.9	6,103	6,369
14/08/15	68	3556	2983	0.8	3047	2660	41	450	148	0.9	5,767	6,770
17/08/15	71	3532	3122	0.9	3250	2833	39	494	152	0.9	6,270	6,579
19/08/15	73	3556	3106	0.9	3087	2710	39	443	144	0.9	6,257	6,968
21/08/15	75	3449	3062	0.9	3353	2940	42	482	144	0.9	6,283	6,957
24/08/15	78	3485	3045	0.9	3313	2900	40	457	138	0.9	6,527	7,250
26/08/15	80	3477	3057	0.9	3163	2650	40	465	147	0.8	6,177	6,803
28/08/15	82	3576	3120	0.9	3250	2710	40	473	146	0.8	6,330	6,871
31/08/15	85	3520	3003	0.9	3077	2627	39	468	152	0.9	6,157	6,574
02/09/15	87	3527	3020	0.9	3373	2960	40	499	148	0.9	6,320	6,760
04/09/15	89	4277	3743	0.9	3570	3027	39	477	134	0.8	7,147	7,484
07/09/15	92	4230	3693	0.9	3827	3277	39	498	130	0.9	7,680	7,684
09/09/15	94	3990	3277	0.8	3690	3170	41	507	137	0.9	6,967	7,278
11/09/15	96	3657	2937	0.8	3780	3283	40	514	136	0.9	6,997	7,354
14/09/15	99	3793	3160	0.8	3483	2960	35	449	129	0.8	7,173	7,758
16/09/15	101	3665	2919	0.8	3603	3070	41	453	126	0.9	6,947	7,954
18/09/15	103	3619	3056	0.8	3350	2843	38	461	138	0.8	6,997	7,267
21/09/15	106	3765	3165	0.8	3500	2977	39	468	134	0.9	7,173	7,479
23/09/15	108	3595	3045	0.8	3317	2790	39	463	140	0.8	7,037	7,163
25/09/15	110	3634	3081	0.8	3360	2817	40	446	133	0.8	6,943	7,534
28/09/15	113	3658	3075	0.8	3557	3033	37	471	132	0.9	6,863	7,551

Table B1.1: Phase II: Sludge data ANX-C, AER-C & RAS MLSS AND MLVSS Cont.

DATE	DAY	ANOXIC MLSS, (mg/L)	ANOXIC MLVSS, (mg/L)	ANOXIC MLVSS/MLSS RATIO	AVERAGE MLSS AERATION (mg/L)	AVERAGE MLVSS AERATION (mg/L)	SRT, (d)	Settled sludge volume, SSV (mL/g)	Sludge Volume Index (SVI), mL/g = (SSV*1000 / MLSS)	AERATION MLVSS / MLSS RATIO	RAS OBSERVED, (mg/L)	RAS PREDICTED, Xr = 10^6/SVI, (mg/L)(mg/L)
28/09/15	113	3658	3075	0.8	3557	3033	37	471	132	0.9	6,863	7,551
30/09/15	115	3753	3227	0.9	3353	2847	37	447	133	0.8	7,173	7,502
02/10/15	117	3609	3036	0.8	3577	3063	40	459	128	0.9	7,037	7,792
05/10/15	120	3672	3092	0.8	3373	2823	41	435	129	0.8	6,917	7,755
07/10/15	122	3681	3101	0.8	3387	2867	40	462	136	0.8	6,937	7,330
09/10/15	124	3760	3120	0.8	3577	3080	41	444	124	0.9	7,307	8,056
12/10/15	127	3782	3179	0.8	3380	2857	39	432	128	0.8	7,297	7,824
14/10/15	129	3690	2997	0.8	3500	2967	41	441	126	0.8	7,190	7,937
16/10/15	131	3648	2988	0.8	3350	2843	37	434	130	0.8	7,537	7,719
19/10/15	134	5427	4757	0.9	3727	3040	38	457	123	0.8	8,557	8,155
21/10/15	136	5147	4673	0.9	4107	3423	39	463	113	0.8	8,127	8,870
23/10/15	138	5393	4930	0.9	3877	3430	37	395	102	0.9	8,913	9,814
26/10/15	141	5193	4570	0.9	3607	3193	30	353	98	0.9	10,160	10,217
28/10/15	143	4863	4393	0.9	3773	3277	29	328	87	0.9	11,230	11,504
30/10/15	145	4949	4409	0.9	3643	3113	30	288	79	0.9	12,237	12,650
02/11/15	148	4803	4199	0.9	3787	3280	30	292	77	0.9	12,513	12,968
04/11/15	150	5049	4362	0.9	3653	3193	29	281	77	0.9	12,887	13,001
06/11/15	152	4927	4247	0.9	3870	3197	31	296	76	0.8	12,433	13,074
09/11/15	155	4997	4274	0.9	4503	3420	33	332	74	0.8	12,577	13,564
11/11/15	157	5132	4356	0.8	5047	3283	39	394	78	0.7	12,270	12,809
13/11/15	159	5077	4203	0.8	5273	3620	40	402	76	0.7	12,623	13,118
16/11/15	162	5184	4384	0.8	5170	3660	37	373	72	0.7	12,637	13,861

Table B1.1: Phase III: Sludge data ANX-C, AER-C & RAS MLSS AND MLVSS

DATE	DAY	ANOXIC MLSS, (mg/L)	ANOXIC MLVSS, (mg/L)	ANOXIC MLVSS/MLSS RATIO	AVERAGE MLSS AERATION (mg/L)	AVERAGE MLVSS AERATION (mg/L)	SRT, (d)	Settled sludge volume, SSV (mL/g)	Sludge Volume Index (SVI), mL/g = (SSV*1000 / MLSS)	AERATION MLVSS / MLSS RATIO	RAS OBSERVED, (mg/L)	RAS PREDICTED, Xr = 10^6/SVI, (mg/L)(mg/L)
16/11/15	162	5184	4384	0.8	5170	3660	37	373	72	0.7	12,637	13,861
18/11/15	164	4068	2915	0.7	5383	3613	44	418	78	0.7	12,770	12,879
20/11/15	166	4089	3077	0.8	5177	3347	40	380	73	0.6	13,013	13,623
23/11/15	169	3729	2777	0.7	5430	3350	41	396	73	0.6	12,733	13,712
25/11/15	171	3814	2836	0.7	5040	3277	43	412	82	0.7	13,133	12,233
27/11/15	173	3479	2625	0.8	5267	2963	42	400	76	0.6	12,500	13,167
30/11/15	176	3306	2442	0.7	4943	2993	42	399	81	0.6	12,940	12,389
02/12/15	178	3331	2503	0.8	5000	3133	41	395	79	0.6	12,483	12,658
04/12/15	180	3413	2477	0.7	4853	2963	41	397	82	0.6	13,343	12,225
07/12/15	183	3319	2366	0.7	5017	3133	40	386	77	0.6	13,170	12,997
09/12/15	185	3327	2386	0.7	4983	2937	40	383	77	0.6	13,340	13,011
11/12/15	187	4012	3079	0.8	5580	3597	36	360	65	0.6	14,093	15,500
14/12/15	190	3856	2929	0.8	5317	3347	37	370	70	0.6	13,177	14,369
16/12/15	192	3605	2929	0.8	5173	3083	35	350	68	0.6	13,877	14,781
18/12/15	194	3904	3267	0.8	5610	3430	40	403	72	0.6	13,453	13,921
21/12/15	197	3883	3189	0.8	5003	2913	33	333	67	0.6	14,147	15,025
23/12/15	199	3599	2773	0.8	5117	3087	36	357	70	0.6	13,477	14,332
25/12/15	201	3501	2884	0.8	5200	3300	35	353	68	0.6	14,180	14,731
28/12/15	204	3576	2869	0.8	5043	2963	36	359	71	0.6	13,903	14,048
30/12/15	206	3459	2913	0.8	4977	3030	36	357	72	0.6	14,147	13,940
01/01/16	208	3436	2832	0.8	5013	3050	37	365	73	0.6	14,010	13,735
04/01/16	211	4348	3878	0.9	5850	3810	34	358	61	0.7	15,060	16,341
06/01/16	213	4766	4139	0.9	5143	3077	34	349	68	0.6	14,547	14,737
08/01/16	215	4362	3705	0.8	5597	3427	33	341	61	0.6	15,100	16,413
11/01/16	218	4494	3800	0.8	5443	3290	32	335	62	0.6	14,700	16,249
13/01/16	220	4596	3819	0.8	5170	3113	32	336	65	0.6	15,297	15,387

Table B1.1: Phase III: Sludge data ANX-C, AER-C & RAS MLSS AND MLVSS cont.

DATE	DAY	ANOXIC MLSS, (mg/L)	ANOXIC MLVSS, (mg/L)	ANOXIC MLVSS/MLSS RATIO	AVERAGE MLSS AERATION (mg/L)	AVERAGE MLVSS AERATION (mg/L)	SRT, (d)	Settled sludge volume, SSV (mL/g)	Sludge Volume Index (SVI), mL/g = (SSV*1000 / MLSS)	AERATION MLVSS / MLSS RATIO	RAS OBSERVED, (mg/L)	RAS PREDICTED, Xr = 10^6/SVI, (mg/L)(mg/L)
18/01/16	225	4474	3781	0.8	4990	3050	30	313	63	0.6	15,320	15,942
20/01/16	227	4563	3859	0.8	5220	3160	32	334	64	0.6	15,680	15,629
22/01/16	229	4479	3899	0.9	5240	3143	31	325	62	0.6	15,640	16,123
25/01/16	232	4477	3807	0.9	5030	2897	29	304	60	0.6	16,027	16,546
27/01/16	234	5329	4609	0.9	5863	3900	26	295	50	0.7	17,620	19,876
29/01/16	236	5521	4681	0.8	5600	3623	28	316	56	0.6	16,997	17,722
01/02/16	239	5003	4459	0.9	5297	3073	25	278	52	0.6	17,870	19,053
03/02/16	241	5596	4743	0.8	5703	3603	24	274	48	0.6	17,287	20,815
05/02/16	243	5127	4577	0.9	5167	3020	25	278	54	0.6	18,213	18,585
08/02/16	246	5219	4443	0.9	5387	3460	25	283	53	0.6	18,117	19,034
10/02/16	248	5206	4429	0.9	5203	3083	25	285	55	0.6	18,167	18,257
12/02/16	250	5105	4489	0.9	5057	2957	24	266	53	0.6	17,877	19,013
15/02/16	253	5310	4650	0.9	5243	3152	26	288	55	0.6	18,037	18,206
17/02/16	255	5236	4356	0.8	5170	3240	25	283	55	0.6	18,337	18,269
19/02/16	257	6199	5359	0.9	5903	3783	23	291	49	0.6	19,803	20,286
22/02/16	260	5787	5054	0.9	5243	3527	19	248	47	0.7	19,480	21,142
24/02/16	262	6114	5314	0.9	5663	3660	23	289	51	0.6	19,067	19,596
26/02/16	264	6058	5641	0.9	5250	3320	20	250	48	0.6	19,800	21,000
29/02/16	267	5989	5299	0.9	5607	3420	21	275	49	0.6	19,367	20,388
02/03/16	269	5815	5222	0.9	5347	3398	20	270	47	0.6	19,317	21,277
04/03/16	271	5974	5111	0.9	5210	3177	20	254	49	0.6	19,707	20,512
07/03/16	274	5784	4984	0.9	5133	3193	20	258	50	0.6	19,560	19,897
09/03/16	276	5878	5101	0.9	5177	3100	20	260	50	0.6	19,433	19,910
11/03/16	278	5755	5178	0.9	5320	3270	20	258	48	0.6	19,727	20,620

Table B1.2: Phase II data for pH, DO and temperature

DATE	DAY	EVENT	DISSOLVED OXYGEN, (mg/L)					pH						TEMPERATURE, °C				
			INFLUENT	ANOXIC CHAMBER	AERATION CHAMBER (Fine bubble diffusers)	AEROBIC DIGESTER (Coarse bubble diffusers)	CLARIFIER	BALANCE TANK (RAW)	INFLUENT - REACTOR	ANOXIC CHAMBER	AERATION CHAMBER	AEROBIC DIGESTER	CLARIFIER EFFLUENT	INFLUENT	ANOXIC CHAMBER	AERATION CHAMBER	AEROBIC DIGESTER	CLARIFIER EFFLUENT
08 Jun 2015	1	START UP, BIOMASS BUILD UP TO 3000, WASTING, AEROBIC DIGESTION		0.26	4.28		0.17	6.3		7.4	7.6		7.4			29.1		
10 Jun 2015	3			0.18	4.82		0.15	5.1		7.6	7.8		7.2			29.5		
12 Jun 2015	5			0.24	4.38		0.15	6.5		7.6	7.8		7.5			29.7		
15 Jun 2015	8			0.26	5.75		0.16	6.2		7.5	7.7		7.3			30.7		
17 Jun 2015	10			0.2	3.77		0.17	6.0		7.4	7.6		7.6			28.2		
19 Jun 2015	12			0.25	4.24		0.17	5.2		7.4	7.6		7.2			29.9		
22 Jun 2015	15			0.19	3.76		0.18	6.3		7.3	7.5		7.5			28.7		
24 Jun 2015	17			0.22	4.50		0.17	6.1		7.5	7.6		7.3			28.8		
26 Jun 2015	19			0.28	4.33		0.17	6.4		7.5	7.6		7.6			29.2		
29 Jun 2015	22			0.24	4.18		0.17	4.5		7.6	7.6		7.4			30.0		
01 Julai 2015	24			0.28	4.64		0.21	4.9		7.4	7.5		7.6			29.2		
03 Julai 2015	26			0.26	4.47		0.17	6.5		7.6	7.6		7.5			29.8		
06 Julai 2015	29			0.24	4.92		0.16	6.2		7.8	7.6		7.6			29.8		
08 Julai 2015	31			0.21	4.38		0.16	5.4		7.4	7.4		7.4			27.3		
10 Julai 2015	33			0.25	4.25		0.16	5.7		7.6	7.5		7.6			28.2		
13 Julai 2015	36			0.23	4.72		0.17	5.2		7.4	7.4		7.4			28.3		
15 Julai 2015	38			0.21	4.86		0.08	5.6		7.6	7.6		7.5			29.1		
17 Julai 2015	40			0.22	4.14		0.07	5.2		7.4	7.5		7.5			28.0		
20 Julai 2015	43			0.20	4.08		0.07	6.3		7.3	7.5		7.5			28.1		
22 Julai 2015	45			0.22	4.63		0.17	4.8		7.5	7.6		7.5			32.1		
24 Julai 2015	47			0.28	4.35		0.18	6.3		7.3	7.5		7.6			29.1		
27 Julai 2015	50			0.22	4.92	7.21	0.16	5.5		7.4	7.5	7.2	7.5			27.6		
29 Julai 2015	52			0.25	4.39	7.66	0.16	5.1		7.3	7.6	7.0	7.6			29.3		
31 Julai 2015	54			0.18	4.41	7.48	0.19	6.2		7.4	7.6	7.1	7.5			29.7		
03 Ogos 2015	57			0.20	4.87	6.93	0.22	5.4		7.5	7.5	7.3	7.5			29.9		
05 Ogos 2015	59			0.22	5.11	7.80	0.18	4.2		7.6	7.6	7.1	7.3			29.0		
07 Ogos 2015	61			0.22	4.83	8.98	0.16	6.3		7.6	7.7	7.0	7.5			28.5		
10 Ogos 2015	64			0.21	4.29	9.05	0.18	6.6		7.4	7.6	7.1	7.7			28.8		
12 Ogos 2015	66			0.20	4.47	8.97	0.21	4.6		7.4	7.6	6.9	7.6			28.3		
14 Ogos 2015	68			0.22	4.74	8.18	0.23	6.2		7.6	7.6	6.9	7.4			29.2		
17 Ogos 2015	71			0.21	4.51	8.80	0.18	4.8		7.4	7.5	7.1	7.3			28.1		
19 Ogos 2015	73			0.28	4.98	7.96	0.16	6.3		7.6	7.6	7.0	7.4			29.8		
21 Ogos 2015	75			0.21	4.34	8.08	0.18	6.1		7.3	7.5	7.1	7.5			29.4		
24 Ogos 2015	78			0.28	4.78	8.63	0.21	6.4		7.4	7.7	7.1	7.6			29.8		
26 Ogos 2015	80			0.25	4.53	8.80	0.15	6.2		7.6	7.4	7.2	7.5			29.6		
28 Ogos 2015	82			0.27	4.32	8.38	0.16	6.6		7.4	7.6	7.0	7.6			29.3		
31 Ogos 2015	85			0.22	4.41	7.95	0.18	4.5		7.3	7.3	7.2	7.5			30.7		
02 September 2015	87			0.24	4.69	9.01	0.19	6.4		7.3	7.5	7.0	7.5			29.5		

Table B1.2: Phase II data for pH, DO and temperature cont.

DATE	DAY	EVENT	DISSOLVED OXYGEN, (mg/L)					pH						TEMPERATURE, °C				
			INFLUENT	ANOXIC CHAMBER	AERATION CHAMBER (Fine bubble diffusers)	AEROBIC DIGESTER (Coarse bubble diffusers)	CLARIFIER	BALANCE TANK (RAW)	INFLUENT - REACTOR	ANOXIC CHAMBER	AERATION CHAMBER	AEROBIC DIGESTER	CLARIFIER EFFLUENT	INFLUENT	ANOXIC CHAMBER	AERATION CHAMBER	AEROBIC DIGESTER	CLARIFIER EFFLUENT
04 September 2015	89	AEROBIC DIGESTION and 24 HRS & INTERNAL RECYCLE OPTIMIZATION (100% vs 50%)		0.26	4.35	8.38	0.15	6.1		7.4	7.6	7.0	7.7			29.6		
07 September 2015	92			0.26	4.88	8.69	0.18	6.2		7.5	7.6	7.1	7.4			28.6		
09 September 2015	94			0.25	4.42	8.07	0.19	6.4		7.3	7.5	6.9	7.7			31.4		
11 September 2015	96			0.25	4.37	9.11	0.22	5.6		7.4	7.6	7.1	7.5			28.7		
14 September 2015	99			0.24	4.93	8.28	0.26	6.3		7.4	7.5	7.2	7.8			29.9		
16 September 2015	101			0.26	4.37	8.36	0.19	5.8		7.3	7.5	7.0	7.6			32.5		
18 September 2015	103			0.22	4.33	7.92	0.23	6.1		7.4	7.6	7.1	7.6			29.9		
21 September 2015	106			0.22	4.84	7.57	0.16	6.5		7.6	7.6	7.2	7.7			29.3		
23 September 2015	108			0.21	5.02	8.15	0.19	6.2		7.4	7.2	7.0	7.5			30.7		
25 September 2015	110			0.24	4.12	7.78	0.16	4.7		7.5	7.6	7.3	7.4			28.6		
28 September 2015	113			0.22	4.51	7.96	0.22	5.9		7.4	7.3	7.0	7.5			32.2		
30 September 2015	115			0.24	4.82	8.39	0.20	5.6		7.7	7.5	7.2	7.7			29.7		
02 Oktober 2015	117			0.22	4.22	7.85	0.18	5.8		7.5	7.4	7.0	7.4			29.6		
05 Oktober 2015	120			0.23	4.46	7.48	0.21	6.4		7.5	7.6	7.1	7.6			29.8		
07 Oktober 2015	122			0.27	4.68	7.6	0.17	4.8		7.3	7.5	7.3	7.4			29.5		
09 Oktober 2015	124			0.25	4.53	7.47	0.19	6.4		7.4	7.4	7.1	7.6			28.6		
12 Oktober 2015	127			0.22	4.09	7.08	0.17	6.6		7.4	7.6	7.0	7.4			29.9		
14 Oktober 2015	129			0.24	4.17	8.39	0.18	6.4		7.6	7.4	7.2	7.6			29.7		
16 Oktober 2015	131			0.26	4.54	7.31	0.19	6.5		7.5	7.6	7.0	7.5			29.9		
19 Oktober 2015	134	BIOMASS BUILD-UP 3000-5000		0.22	4.77	7.80	0.19	6.2		7.4	7.7	7.1	7.3			28.5		
21 Oktober 2015	136			0.24	4.39	8.39	0.20	6.1		7.6	7.4	7.0	7.6			29.2		
23 Oktober 2015	138			0.22	4.07	8.58	0.20	6.4		7.3	7.7	7.2	7.6			28.8		
26 Oktober 2015	141			0.25	4.32	8.97	0.19	5.3		7.5	7.5	7.0	7.4			29.5		
28 Oktober 2015	143			0.21	4.41	8.18	0.16	6.2		7.4	7.6	7.0	7.8			32.7		
30 Oktober 2015	145			0.22	4.79	8.81	0.19	6.4		7.6	7.6	7.0	7.5			28.5		
02 November 2015	148			0.22	4.41	8.21	0.20	6.3		7.4	7.7	7.2	7.6			28.8		
04 November 2015	150			0.23	4.33	8.35	0.22	6.5		7.5	7.6	7.1	7.7			27.9		
06 November 2015	152			0.26	4.55	8.08	0.20	6.8		7.3	7.5	7.3	7.6			28.2		
09 November 2015	155			0.26	4.12	8.24	0.19	6.6		7.4	7.4	7.1	7.5			28.7		
11 November 2015	157			0.23	4.92	8.67	0.19	6.3		7.4	7.6	7.0	7.6			29.5		
13 November 2015	159			0.27	4.06	8.38	0.15	6.5		7.6	7.5	7.3	7.5			29.8		
16 November 2015	162			0.24	4.36	8.89	0.24	6.2		7.5	7.6	7.1	7.6			28.9		
18 November 2015	164			0.26	4.71	8.98	0.25	5.7		7.6	7.6	7.3	7.5			29.7		
20 November 2015	166			0.28	4.16	8.36	0.22	6.4		7.4	7.6	7.1	7.7			29.5		
23 November 2015	169			0.26	4.43	7.97	0.25	5.1		7.5	7.6	7.0	7.4			29.3		
25 November 2015	171			0.26	4.86	9.29	0.23	6.4		7.4	7.5	7.2	7.5			30.1		
27 November 2015	173			0.25	4.08	9.33	0.24	6.1		7.2	7.4	7.2	7.6			30.4		
30 November 2015	176			0.27	4.62	8.38	0.25	5.9		7.4	7.6	7	7.5			28.8		

Table B1.2: Phase III data for pH, DO and temperature

DATE	DAY	EVENT	DISSOLVED OXYGEN, (mg/L)						pH						TEMPERATURE, °C				
			INFLUENT	ANOXIC CHAMBER	AERATION CHAMBER (Fine bubble diffusers)	AEROBIC DIGESTER (Coarse bubble diffusers)	CLARIFIER		BALANCE TANK (RAW)	INFLUENT - REACTOR	ANOXIC CHAMBER	AERATION CHAMBER	AEROBIC DIGESTER	CLARIFIER EFFLUENT	INFLUENT	ANOXIC CHAMBER	AERATION CHAMBER	AEROBIC DIGESTER	CLARIFIER EFFLUENT
18 Disember 2015	194	KINETIC STUDY PERIOD (PHASE II TO PHASE III FLOW RATE 180 L/HR TO 120 L/HR)		0.24	4.12	8.59	0.17		5.5		7.3	7.5	6.9	7.7			29.6		
21 Disember 2015	197			0.27	4.76	8.87	0.17		5.2		7.4	7.6	7.1	7.8			30.7		
23 Disember 2015	199			0.25	4.39	8.21	0.16		6.1		7.4	7.5	7.2	7.5			29.4		
25 Disember 2015	201			0.22	4.52	8.48	0.16		5.8		7.3	7.5	7.1	7.6			29.6		
28 Disember 2015	204			0.25	4.87	8.26	0.16		5.9		7.4	7.6	7.1	7.6			28.8		
30 Disember 2015	206			0.23	4.28	8.19	0.17		6.0		7.6	7.6	7.2	7.7			29.5		
01 Januari 2016	208			0.27	4.73	7.77	0.27		5.8		7.4	7.2	7.0	7.5			28.7		
04 Januari 2016	211			0.22	4.47	7.98	0.20		5.9		7.5	7.5	6.8	7.4			29.5		
06 Januari 2016	213			0.22	4.23	8.36	0.20		6.2		7.4	7.3	7.0	7.6			29.8		
08 Januari 2016	215			0.25	4.76	7.87	0.20		5.9		7.7	7.5	7.2	7.3			30.2		
11 Januari 2016	218			0.21	4.63	7.49	0.21		6.0		7.5	7.4	7.0	7.4			29.8		
13 Januari 2016	220			0.23	4.36	7.62	0.19		5.9		7.4	7.2	7.3	7.6			30.6		
15 Januari 2016	222			0.25	4.13	8.18	0.19		6.2		7.3	7.4	7.1	7.5			28.3		
18 Januari 2016	225			0.26	4.59	7.51	0.19		6.3		7.4	7.6	7.1	7.7			29.8		
20 Januari 2016	227			0.23	4.22	7.68	0.19		5.8		7.6	7.4	7.2	7.4			28.6		
22 Januari 2016	229			0.25	3.98	7.98	0.20		6.3		7.3	7.3	7.0	7.5			29.8		
25 Januari 2016	232			0.22	4.77	7.72	0.21		4.4		7.5	7.4	7.2	7.7			29.9		
27 Januari 2016	234			0.21	4.22	8.98	0.20		6.2		7.4	7.2	7.0	7.4			29.8		
29 Januari 2016	236			0.22	4.59	7.52	0.20		6.4		7.5	7.5	7.0	7.5			29.7		
01 Februari 2016	239			0.24	4.08	8.97	0.19		5.3		7.5	7.5	7.1	7.4			29.8		
03 Februari 2016	241			0.22	4.18	8.19	0.20		6.2		7.6	7.6	7.0	7.6			30.4		
05 Februari 2016	243			0.21	4.63	8.49	0.18		6.2		7.3	7.5	7.1	7.3			29.6		
08 Februari 2016	246			0.22	4.41	7.68	0.20		6.6		7.6	7.3	7.2	7.5			29.8		
10 Februari 2016	248			0.25	4.78	8.41	0.19		5.6		7.4	7.6	7.0	7.4			28.6		
12 Februari 2016	250			0.21	4.21	8.08	0.19		4.9		7.6	7.6	7.1	7.6			29.9		
15 Februari 2016	253			0.23	4.48	9.05	0.18		4.5		7.5	7.5	7.2	7.8			29.7		
17 Februari 2016	255			0.26	4.69	8.68	0.20		6.4		7.4	7.6	7.0	7.4			30.9		
19 Februari 2016	257			0.24	4.09	8.57	0.19		6.2		7.6	7.4	6.9	7.7			30.4		
22 Februari 2016	260			0.22	4.17	8.78	0.18		5.5		7.4	7.6	7.1	7.6			32.9		
24 Februari 2016	262			0.25	4.41	8.39	0.19		6.3		7.5	7.4	7.2	7.5			31.3		
26 Februari 2016	264			0.22	4.23	8.32	0.20		4.5		7.3	7.6	7.1	7.6			30.7		
29 Februari 2016	267			0.23	4.53	8.85	0.20		6.4		7.6	7.5	7.1	7.5			29.8		
02 Mac 2016	269			0.25	4.15	8.39	0.20		5.2		7.4	7.6	7.2	7.3			32.2		
04 Mac 2016	271			0.27	4.37	8.67	0.19		6.5		7.6	7.5	7.0	7.5			30.4		
07 Mac 2016	274			0.25	4.71	8.26	0.20		6.1		7.5	7.6	6.8	7.7			30.5		
09 Mac 2016	276			0.22	4.18	8.68	0.19		5.4		7.4	7.6	7.0	7.4			30.8		
11 Mac 2016	278			0.23	4.87	8.29	0.18		4.8		7.6	7.4	7.1	7.5			33.3		

APPENDIX C

AD-C AND WASTING RATE MASS BALANCE

Table C1.1: Phase II data for solids mass balance and underflow

Day	AD MLSS, (mg/L)	EAD MLSS, (mg/L)	AD MLVSS, (mg/L)	EAD MLVSS, (mg/L)	AD MLVSS/MLSS ratio	EAD MLVSS/MLSS ratio	Vol. AD-C (L)	Flow rate to AD-C (L/d)	AD Mass, (mg/d)	EAD Mass, (mg/d)	AD-C MLSS, (mg/L)	AD-C MLVSS, (mg/L)	AD-C MLVSS/MLSS ratio	AD-C MLSS Accum. (mg/L)	Solids destroyed, (mg/d)	% Solids destroyed	Wasting rate, (mg/d)	Qw, (L/d)	SSGBR SRT, (d)	Mass of actual waste, (mg/d)	Actual waste, (L/d)
	(A)	(B)	(C)	(D)	(E)=C/A	(F)=D/B	(G)	(H)	(J)=A*7.5	(K)=B*7.5	(L)	(M)	(N)=M/L	(P)=M$_{n-1}$-M$_n$	(Q)=J$_{n-1}$-K-(P*75)	(R)=(Q/J$_{n-1}$)*100	(S)=A$_{ss}$*T	(T)=(VX)/(θ$_c$*A)	(U)=(V*X)/(Q$_w$*A)	(Y)=(S)-(Q)	(Y)/(A)
50	6,867		5,350		0.78				51,500		8,145	5,613	0.69					1.5	37.6		
52	6,323	4,847	4,940	3,193	0.78	0.66	75	7.5	47,425	36,350	8,138	5,647	0.69	(6.7)	15,650	30	10,300	1.5	39.7	(5350)	(0.8)
54	6,873	5,167	5,377	3,560	0.78	0.69	75	7.5	51,550	38,750	8,128	5,630	0.69	(10.0)	9,425	20	9,485	1.5	41.5	60	0.0
57	6,330	5,037	4,917	4,000	0.78	0.79	75	7.5	47,475	37,775	8,138	5,633	0.69	10.0	13,025	25	10,997	1.6	40.4	(2028)	(0.3)
59	5,877	4,240	4,350	3,470	0.74	0.82	75	7.5	44,075	31,800	8,142	5,640	0.69	3.3	15,425	32	10,128	1.6	41.3	(5297)	(0.9)
61	6,123	4,733	4,627	2,560	0.76	0.54	75	7.5	45,925	35,500	8,128	5,630	0.69	(13.3)	9,575	22	9,403	1.6	36.2	(172)	(0.0)
64	5,780	4,420	4,243	2,657	0.73	0.60	75	7.5	43,350	33,150	8,138	5,633	0.69	10.0	12,025	26	9,797	1.6	42.8	(2228)	(0.4)
66	6,103	5,080	4,670	3,633	0.77	0.72	75	7.5	45,775	38,100	8,122	5,617	0.69	(16.7)	6,500	15	8,670	1.5	38.3	2170	0.4
68	5,767	4,220	4,213	2,407	0.73	0.57	75	7.5	43,250	31,650	8,125	5,610	0.69	3.3	13,875	30	9,765	1.6	41.3	(4110)	(0.7)
71	6,270	4,823	4,670	3,023	0.74	0.63	75	7.5	47,025	36,175	8,122	5,603	0.69	(3.3)	7,325	17	9,515	1.7	39.3	2190	0.3
73	6,257	4,553	4,637	2,453	0.74	0.54	75	7.5	46,925	34,150	8,128	5,607	0.69	6.7	12,375	26	10,032	1.6	38.5	(2343)	(0.4)
75	6,283	5,173	4,823	3,227	0.77	0.62	75	7.5	47,125	38,800	8,122	5,610	0.69	(6.7)	8,625	18	10,011	1.6	41.7	1386	0.2
78	6,527	5,010	4,833	3,193	0.74	0.64	75	7.5	48,950	37,575	8,125	5,617	0.69	3.3	9,300	20	10,053	1.6	39.7	753	0.1
80	6,177	5,170	5,007	3,383	0.81	0.65	75	7.5	46,325	38,775	8,138	5,623	0.69	13.3	9,175	19	10,443	1.6	40.0	1268	0.2
82	6,330	4,927	4,723	3,320	0.75	0.67	75	7.5	47,475	36,950	8,145	5,627	0.69	6.7	8,875	19	9,883	1.6	40.1	1008	0.2
85	6,157	4,890	4,793	3,353	0.78	0.69	75	7.5	46,175	36,675	8,162	5,637	0.69	16.7	9,550	20	10,128	1.6	39.0	578	0.1
87	6,320	5,033	4,610	3,190	0.73	0.63	75	7.5	47,400	37,750	8,152	5,640	0.69	(10.0)	9,175	20	10,159	1.7	40.4	984	0.2

Table C1.1: Phase II data for solids mass balance and underflow cont.

Day	MD MLSS, (mg/L)	EAD MLSS, (mg/L)	MD MLVSS, (mg/L)	EAD MLVSS, (mg/L)	MD MLVSS/MLSS ratio	EAD MLVSS/MLSS ratio	Vol. AD-C (L)	Flow rate to AD-C (L/d)	MD Mass, (mg/d)	EAD Mass, (mg/d)	AD-C MLSS, (mg/L)	AD-C MLVSS, (mg/L)	AD-C MLVSS/MLSS ratio	AD-C MLSS Accum. (mg/L)	Solids destroyed, (mg/d)	% Solids destroyed	Wasting rate, (mg/d)	Q_W, (L/d)	ASGBR SRT, (d)	Mass of actual waste, (mg/d)	Actual waste, (L/d)
	(A)	(B)	(C)	(D)	(E)=C/A	(F)=D/B	(G)	(H)	(J)=A*7.5	(K)=B*7.5	(L)	(M)	(N)=M/L	(P)=M_{n-1}-M	(Q)=J_{n-1}-K-(P*75)	(R)=(Q/J_{n-1})*100	(S)=A_{n-1}*T	(T)=(VX)/(E_{n-1}*A)	(U)=(V*X)/(Q_W*A)	(Y)=(S)-(Q)	(Y)/(A)
85	6,157	4,890	4,793	3,353	0.78	0.69	75	7.5	46,175	36,675	8,162	5,637	0.69	16.7	9,550	20	10,128	1.6	39.0	578	0.1
87	6,320	5,033	4,610	3,190	0.73	0.63	75	7.5	47,400	37,750	8,152	5,640	0.69	(10.0)	9,175	20	10,159	1.7	40.4	984	0.2
89	7,147	5,377	5,093	4,320	0.71	0.80	75	7.5	53,600	40,325	8,138	5,643	0.69	(13.3)	8,075	17	10,112	1.6	39.0	2037	0.3
92	7,680	6,233	5,580	3,483	0.73	0.56	75	7.5	57,600	46,750	8,132	5,637	0.69	(6.7)	7,350	14	11,435	1.6	38.9	4085	0.5
94	6,967	6,200	5,213	4,060	0.75	0.65	75	7.5	52,250	46,500	8,145	5,643	0.69	13.3	10,100	18	12,288	1.6	41.4	2188	0.3
96	6,997	5,673	5,303	4,250	0.76	0.75	75	7.5	52,475	42,550	8,155	5,640	0.69	10.0	8,950	17	11,147	1.6	40.2	2197	0.3
99	7,173	5,617	5,277	3,573	0.74	0.64	75	7.5	53,800	42,125	8,162	5,630	0.69	6.7	9,850	19	11,195	1.6	35.1	1345	0.2
101	6,947	5,897	5,113	4,277	0.74	0.73	75	7.5	52,100	44,225	8,145	5,637	0.69	(16.7)	10,825	20	11,477	1.6	40.5	652	0.1
103	6,997	5,743	5,303	3,950	0.76	0.69	75	7.5	52,475	43,075	8,152	5,650	0.69	6.7	8,525	16	10,420	1.5	38.4	1895	0.3
106	7,173	5,787	5,277	4,163	0.74	0.72	75	7.5	53,800	43,400	8,148	5,657	0.69	(3.3)	9,325	18	10,495	1.5	39.0	1170	0.2
108	7,037	6,120	5,037	4,017	0.72	0.66	75	7.5	52,775	45,900	8,138	5,647	0.69	(10.0)	8,650	16	10,760	1.5	38.6	2110	0.3
110	6,943	5,760	5,030	3,873	0.72	0.67	75	7.5	52,075	43,200	8,148	5,657	0.69	10.0	8,825	17	10,555	1.5	40.3	1730	0.2
113	6,863	5,860	5,170	3,800	0.75	0.65	75	7.5	51,475	43,950	8,145	5,643	0.69	(3.3)	8,375	16	11,109	1.6	36.8	2734	0.4
115	7,173	5,723	5,277	3,697	0.74	0.65	75	7.5	53,800	42,925	8,142	5,630	0.69	(3.3)	8,800	17	10,981	1.6	36.5	2181	0.3
117	7,037	5,987	5,037	3,963	0.72	0.66	75	7.5	52,775	44,900	8,148	5,617	0.69	6.7	8,400	16	11,477	1.6	39.7	3077	0.4
120	6,917	5,847	5,123	4,067	0.74	0.70	75	7.5	51,875	43,850	8,145	5,630	0.69	(3.3)	9,175	17	10,555	1.5	40.6	1380	0.2
122	6,997	5,773	5,303	3,860	0.76	0.67	75	7.5	52,475	43,300	8,148	5,623	0.69	3.3	8,325	16	10,375	1.5	40.3	2050	0.3
124	7,307	5,823	5,403	4,123	0.74	0.71	75	7.5	54,800	43,675	8,145	5,620	0.69	(3.3)	9,050	17	10,495	1.5	40.8	1445	0.2
127	7,297	6,093	5,490	4,243	0.75	0.70	75	7.5	54,725	45,700	8,148	5,630	0.69	3.3	8,850	16	10,960	1.5	38.6	2110	0.3
129	7,190	6,070	5,293	4,360	0.74	0.72	75	7.5	53,925	45,525	8,162	5,623	0.69	13.3	8,200	15	10,945	1.5	40.6	2745	0.4

Table C1.1: Phase II data for solids mass balance and underflow cont.

Day	AD MLSS, (mg/L)	EAD MLSS, (mg/L)	AD MLVSS, (mg/L)	EAD MLVSS, (mg/L)	AD MLVSS/MLSS ratio	EAD MLVSS/MLSS ratio	Vol. AD-C (L)	Flow rate to AD-C (L/d)	AD Mass, (mg/d)	EAD Mass, (mg/d)	AD-C MLSS, (mg/L)	AD-C MLVSS, (mg/L)	AD-C MLVSS/MLSS ratio	AD-C MLSS Accum (mg/L)	Solids destroyed, (mg/d)	% Solids destroyed	Wasting rate, (mg/d)	Qw, (L/d)	I-SGBR SRT, (d)	Mass of actual waste, (mg/d)	Actual waste, (L/d)
	(A)	(B)	(C)	(D)	(E)=C/A	(F)=D/B	(G)	(H)	(I)=A*7.5	(K)=B*7.5	(L)	(M)	(N)=M/L	(P)=M$_{n-1}$-M$_n$	(R)=L$_{n-1}$-K-(P*75)	(R)=(R)*100	(S)=A$_{n-1}$*T	(T)=(VX)/(E$_{n-1}$*A)	(U)=(V*X)/(Q$_{n-1}$*A)	(Y)=(S)-(Q)	(Y)/(A)
127	7,297	6,093	5,490	4,243	0.75	0.70	75	7.5	54,725	45,700	8,148	5,630	0.69	3.3	8,850	16	10,960	1.5	38.6	2110	0.3
129	7,190	6,070	5,293	4,360	0.74	0.72	75	7.5	53,925	45,525	8,162	5,623	0.69	13.3	8,200	15	10,945	1.5	40.6	2745	0.4
131	7,537	6,157	5,827	4,133	0.77	0.67	75	7.5	56,525	46,175	8,148	5,633	0.69	(13.3)	8,750	16	10,785	1.5	37.0	2035	0.3
134	8,557	6,873	6,523	5,163	0.76	0.75	75	7.5	64,175	51,550	8,142	5,640	0.69	(6.7)	5,475	10	11,305	1.5	38.1	5830	0.7
136	8,127	7,167	6,823	5,657	0.84	0.79	75	7.5	60,950	53,750	8,135	5,653	0.69	(6.7)	10,925	17	13,631	1.6	39.5	2766	0.3
138	8,913	7,150	7,080	5,613	0.79	0.79	75	7.5	66,850	53,625	8,125	5,663	0.70	(10.0)	8,075	13	11,784	1.5	37.5	3709	0.4
141	10,160	7,620	8,847	6,190	0.87	0.81	75	7.5	76,200	57,150	8,112	5,650	0.70	(13.3)	10,700	16	12,924	1.5	30.4	2224	0.2
143	11,230	8,570	8,547	7,073	0.76	0.83	75	7.5	84,225	64,275	8,098	5,643	0.70	(13.3)	12,925	17	14,732	1.5	29.0	1807	0.2
145	12,237	10,253	9,743	7,283	0.80	0.71	75	7.5	91,775	76,900	8,095	5,630	0.70	(3.3)	7,575	9	13,476	1.2	30.0	5901	0.5
148	12,513	10,380	9,523	7,360	0.76	0.71	75	7.5	93,850	77,850	8,102	5,627	0.69	6.7	13,425	15	14,684	1.2	30.4	1259	0.10
150	12,987	10,550	10,167	7,350	0.79	0.70	75	7.5	96,650	79,125	8,115	5,637	0.69	13.3	13,725	15	15,016	1.2	29.3	1291	0.10
152	12,433	11,093	9,710	7,863	0.78	0.71	75	7.5	93,250	83,200	8,108	5,630	0.69	(6.7)	13,950	14	15,464	1.2	30.8	1514	0.12
155	12,577	10,603	10,600	7,543	0.84	0.71	75	7.5	94,325	79,525	8,118	5,620	0.69	10.0	12,975	14	15,542	1.3	33.2	2567	0.20
157	12,270	10,823	10,460	8,357	0.85	0.77	75	7.5	92,025	81,175	8,108	5,633	0.69	(10.0)	13,900	15	15,721	1.3	39.4	1821	0.15
159	12,623	10,393	10,640	8,230	0.84	0.79	75	7.5	94,675	77,950	8,118	5,640	0.69	9.3	13,375	15	15,338	1.3	40.2	1962	0.16
162	12,637	10,923	10,640	8,360	0.84	0.77	75	7.5	94,775	81,925	8,108	5,650	0.70	(9.3)	13,450	14	15,779	1.3	37.3	2329	0.18

Table C1.2: Phase III data for solids mass balance and underflow cont.

Day	AD MLSS, (mg/L)	EAD MLSS, (mg/L)	AD MLVSS, (mg/L)	EAD MLVSS, (mg/L)	AD MLVSS/MLSS ratio	EAD MLVSS/MLSS ratio	Vol. AD-C (L)	Flow rate to AD-C (L/d)	AD Mass, (mg/d)	EAD Mass, (mg/d)	AD-C MLSS, (mg/L)	AD-C MLVSS, (mg/L)	AD-C MLVSS/MLSS ratio	AD-C MLSS Accum. (mg/L)	Solids destroyed, (mg/d)	% Solids destroyed	Wasting rate, (mg/d)	Qw, (L/d)	ASGBR SRT, (d)	Mass of actual waste, (mg/d)	Actual waste, (L/d)
	(A)	(B)	(C)	(D)	(E)=C/A	(F)=D/B	(G)	(H)	(J)=A*7.5	(K)=B*7.5	(L)	(M)	(N)=M/L	(P)=M_{n-1}-M	(Q)=J_{n-1}-K-(P*75)	(R)=(Q/J_{n-1})*100	(S)=A_{n-1}*T	(T)=(VX)/(S_e*A)	(U)=(V*X)/(Q_w*A)	(V)=(S)-(Q)	(Y)=(V)/(A)
162	12,637	10,923	10,640	8,360	0.84	0.77	75	7.5	94,775	81,925	8,108	5,650	0.70	(9.3)	13,450	14	15,779	1.3	37.3	2329	0.18
164	12,770	11,157	10,727	9,053	0.84	0.81	75	7.5	95,775	83,675	8,071	5,643	0.70	(37.3)	13,900	15	15,164	1.2	43.5	1264	0.10
166	13,013	11,600	10,413	8,953	0.80	0.77	75	7.5	97,600	87,000	8,048	5,653	0.70	(22.7)	10,475	11	15,324	1.2	39.6	4849	0.37
169	12,733	11,350	10,697	9,253	0.84	0.82	75	7.5	95,500	85,125	8,065	5,667	0.70	16.7	11,225	12	15,616	1.2	41.3	4391	0.34
171	13,133	11,083	11,013	8,910	0.84	0.80	75	7.5	98,500	83,125	8,058	5,660	0.70	(6.7)	12,875	13	15,280	1.2	42.9	2405	0.18
173	12,500	11,283	10,453	9,207	0.84	0.82	75	7.5	93,750	84,625	8,052	5,647	0.70	(6.7)	14,375	15	15,760	1.2	41.7	1385	0.11
176	12,940	10,607	10,773	8,383	0.83	0.79	75	7.5	97,050	79,550	8,062	5,633	0.70	10.0	13,450	14	15,000	1.2	41.6	1550	0.12
178	12,493	11,050	10,387	8,940	0.83	0.81	75	7.5	93,625	82,875	8,048	5,640	0.70	(13.3)	15,175	16	15,528	1.2	41.1	353	0.03
180	13,343	10,970	11,210	8,653	0.84	0.79	75	7.5	100,075	82,275	8,015	5,630	0.70	(33.3)	13,850	15	14,980	1.2	41.4	1130	0.08
183	13,170	11,207	11,020	9,303	0.84	0.83	75	7.5	98,775	84,050	8,042	5,627	0.70	26.7	14,025	14	16,012	1.2	40.2	1987	0.15
185	13,340	11,127	11,077	8,670	0.83	0.78	75	7.5	100,050	83,450	8,082	5,633	0.70	40.0	12,325	12	15,804	1.2	39.9	3479	0.26
187	14,093	11,593	11,893	9,480	0.84	0.82	75	7.5	105,700	86,950	8,048	5,627	0.70	(33.3)	15,600	16	16,675	1.3	36.0	1075	0.08
190	13,177	11,553	10,957	8,830	0.83	0.76	75	7.5	98,825	86,650	8,038	5,613	0.70	(10.0)	19,800	19	17,617	1.3	37.0	(2183)	(0.17)
192	13,877	12,150	11,653	10,117	0.84	0.83	75	7.5	104,075	91,125	8,045	5,603	0.70	6.7	7,200	7	16,471	1.3	35.0	9271	0.67
194	13,453	11,757	11,173	9,410	0.83	0.80	75	7.5	100,900	88,175	8,052	5,590	0.69	6.7	15,400	15	17,346	1.3	40.3	1946	0.14
197	14,147	12,613	11,780	10,237	0.83	0.81	75	7.5	106,100	94,600	8,038	5,583	0.69	(13.3)	7,300	7	16,817	1.3	33.3	9517	0.67
199	13,477	12,260	11,243	10,017	0.83	0.82	75	7.5	101,075	91,950	8,042	5,593	0.70	3.3	13,900	13	17,683	1.3	35.7	3783	0.28
201	14,180	12,277	11,857	9,767	0.84	0.80	75	7.5	106,350	92,075	7,975	5,600	0.70	(66.7)	14,000	14	16,846	1.3	35.3	2846	0.20
204	13,903	12,137	11,520	9,623	0.83	0.79	75	7.5	104,275	91,025	8,035	5,590	0.70	60.0	10,825	10	17,725	1.3	35.9	6900	0.50
206	14,147	12,127	11,780	9,677	0.83	0.80	75	7.5	106,100	90,950	8,012	5,607	0.70	(23.3)	15,075	14	17,379	1.3	35.7	2304	0.16
208	14,010	12,243	11,780	9,537	0.84	0.78	75	7.5	105,075	91,825	8,022	5,593	0.70	10.0	13,525	13	17,683	1.3	36.5	4158	0.30
211	15,060	12,783	13,143	10,210	0.87	0.80	75	7.5	112,950	95,875	8,032	5,610	0.70	10.0	8,450	8	18,213	1.3	34.4	9763	0.65

Table C1.2: Phase III data for solids mass balance and underflow cont.

Day	AD VLSS (mg/L)	EAD VLSS, (mg/L)	IAD VLVSS, (mg/L)	EAD VLVSS, (mg/L)	AD VLVSS/VLSS ratio	EAD VLVSS/VLSS ratio	% AD-C (L)	Flow rate to AD-C (L/h)	AD Mass, (mg/d)	EAD Mass, (mg/d)	AD-C VLSS, (mg/L)	AD-C MLVSS (mg/L)	AD-C MLVSS/VLSS ratio	AD-C MLSS Accum. (mg/L)	Solids destroyed (mg/d)	% Solids destroyed	Wasting rate, (mg/d)	Gov. (L/d)	ASQP SRT (d)	Mass of actual waste (mg/d)	Actual waste, (L/d)
211	15,060	12,783	13,143	10,210	0.87	0.80	75	7.5	112,950	95,875	8,032	5,610	0.70	10.0	8,450	8	18,213	1.3	34.4	9763	0.65
213	14,547	12,177	12,480	9,657	0.86	0.79	75	7.5	109,100	91,325	8,042	5,617	0.70	10.0	20,875	18	19,578	1.3	33.6	(1297)	(0.09)
215	15,100	13,147	12,730	10,960	0.84	0.83	75	7.5	113,250	98,600	8,022	5,607	0.70	(20.0)	12,000	11	18,911	1.3	32.8	6911	0.46
218	14,700	12,667	12,347	10,270	0.84	0.81	75	7.5	110,250	95,000	8,018	5,600	0.70	(3.3)	18,500	16	19,630	1.3	32.2	1130	0.08
220	15,297	12,453	13,330	9,573	0.87	0.77	75	7.5	114,725	93,400	8,015	5,590	0.70	(3.3)	17,100	16	19,110	1.3	32.3	2010	0.13
222	15,443	13,827	12,827	10,577	0.83	0.76	75	7.5	115,825	103,700	8,005	5,583	0.70	(10.0)	11,775	10	19,886	1.3	31.2	8111	0.53
225	15,320	13,600	12,967	10,960	0.85	0.81	75	7.5	114,900	102,000	8,012	5,587	0.70	6.7	13,325	12	20,076	1.3	30.1	6751	0.44
227	15,680	13,803	12,880	10,870	0.82	0.79	75	7.5	117,600	103,525	7,988	5,577	0.70	(23.3)	13,125	11	19,916	1.3	32.1	6791	0.43
229	15,640	14,070	13,290	10,973	0.85	0.78	75	7.5	117,300	105,525	7,982	5,563	0.70	(6.7)	12,575	11	20,384	1.3	31.3	7809	0.50
232	16,027	14,083	13,600	11,420	0.85	0.81	75	7.5	120,200	105,625	7,955	5,580	0.70	(26.7)	13,675	12	20,332	1.3	29.2	6657	0.42
234	17,620	14,183	14,110	12,347	0.80	0.87	75	7.5	132,150	106,375	8,032	5,593	0.70	76.7	8,075	7	22,437	1.4	26.3	14362	0.82
236	16,997	14,920	13,437	11,550	0.79	0.77	75	7.5	127,475	111,900	8,045	5,580	0.69	13.3	19,250	15	24,668	1.4	28.2	5418	0.32
239	17,870	15,687	14,203	12,103	0.79	0.77	75	7.5	134,025	117,650	8,025	5,583	0.70	(20.0)	11,325	9	23,795	1.4	24.8	12470	0.70
241	17,287	15,417	13,787	11,450	0.80	0.74	75	7.5	129,650	115,625	8,008	5,567	0.70	(16.7)	19,650	15	25,018	1.4	24.5	5368	0.31
243	18,213	15,810	14,347	12,147	0.79	0.77	75	7.5	136,600	118,575	7,988	5,553	0.70	(20.0)	12,575	10	24,201	1.4	24.8	11626	0.64
246	18,117	16,510	14,610	13,053	0.81	0.79	75	7.5	135,875	123,825	7,982	5,563	0.70	(6.7)	13,275	10	25,499	1.4	25.3	12224	0.67
248	18,167	17,077	14,660	12,513	0.81	0.73	75	7.5	136,250	128,075	7,948	5,547	0.70	(33.3)	10,300	8	25,363	1.4	25.4	15063	0.83
250	17,877	16,613	14,587	13,007	0.82	0.78	75	7.5	134,075	124,600	7,955	5,533	0.70	6.7	11,150	8	25,433	1.4	23.8	14283	0.80
253	18,037	16,473	14,570	12,897	0.81	0.78	75	7.5	135,275	123,550	7,932	5,527	0.70	(23.3)	12,275	9	25,027	1.4	26.7	12752	0.71
255	18,337	16,567	14,603	12,933	0.80	0.78	75	7.5	137,525	124,250	7,928	5,543	0.70	(3.3)	11,275	8	25,251	1.4	25.3	13976	0.76
257	19,803	17,237	15,383	13,573	0.78	0.79	75	7.5	148,525	129,275	7,878	5,557	0.71	(50.0)	12,000	9	29,339	1.6	22.7	17339	0.88
260	19,480	16,683	15,280	12,577	0.78	0.75	75	7.5	146,100	125,125	7,908	5,547	0.70	30.0	21,150	14	31,685	1.6	19.4	10535	0.54
262	19,067	17,363	14,663	13,347	0.77	0.77	75	7.5	143,000	130,225	7,882	5,540	0.70	(26.7)	17,875	12	31,168	1.6	22.6	13293	0.70
264	19,800	17,263	15,413	12,163	0.78	0.70	75	7.5	148,500	129,475	7,812	5,550	0.71	(70.0)	18,775	13	30,507	1.6	19.5	11732	0.59
267	19,367	18,120	14,920	14,037	0.77	0.77	75	7.5	145,250	135,900	7,875	5,537	0.70	63.3	7,950	5	31,680	1.6	21.5	23830	1.23
269	19,317	18,023	14,950	13,530	0.77	0.75	75	7.5	144,875	135,175	7,852	5,547	0.71	(23.3)	11,825	8	30,987	1.6	19.6	19162	0.99
271	19,707	18,280	15,327	13,457	0.78	0.74	75	7.5	147,800	137,100	7,822	5,533	0.71	(30.0)	10,025	7	30,907	1.6	19.8	20882	1.06
274	19,560	18,540	15,193	13,697	0.78	0.74	75	7.5	146,700	139,050	7,792	5,543	0.71	(30.0)	11,000	7	31,531	1.6	20.2	20531	1.05
276	19,433	18,460	14,950	13,697	0.77	0.74	75	7.5	145,750	138,450	7,762	5,527	0.71	(40.0)	11,250	8	31,296	1.6	20.3	20046	1.03
278	19,727	18,323	15,160	13,610	0.77	0.74	75	7.5	-	137,425	7,742	5,540	0.72	(10.0)	9,075	6	31,093	1.6	20.2	22018	1.12

APPENDIX D

DATA FOR i-SGBR PERFORMANCE

Table D1.1: Phase II data for COD and sCOD

OPERATIONAL DAY	INFLUENT AVERAGE COD (mg/L)	INFLUENT AVERAGE sCOD (mg/L)	ANOXIC EFFLUENT COD (mg/L)	ANOXIC EFFLUENT sCOD (mg/L)	AERATION EFFLUENT sCOD (mg/L)	RAS sCOD = INFL DIGESTE= (mg/L)	CLARIFIER EFFLUENT sCOD (mg/L)	CLARIFIER EFFLUENT COD (mg/L)
1	1143	469	559	250	70	80	52	47
3	1967	360	611	272	115	110	105	450
5	1151	346	636	284	124	122	103	427
8	535	360	503	211	49	55	58	230
10	1055	436	525	255	62	64	70	220
12	1125	418	636	267	74	77	58	224
15	1030	456	630	252	50	55	45	203
17	593	390	505	252	65	55	47	156
19	1035	415	555	210	50	65	50	45
22	921	342	504	222	51	54	40	31
24	1961	515	575	229	51	51	50	53
26	1110	445	543	220	59	55	40	25
29	1955	515	597	234	52	65	45	50
31	1035	454	550	227	56	51	44	25
33	1534	360	637	295	66	55	47	27
35	1116	440	545	223	61	60	45	24
38	1455	350	515	241	67	65	47	25
40	1445	515	593	295	69	51	45	23
43	593	456	541	223	69	55	45	29
45	1425	304	589	283	67	65	46	23
47	1415	450	574	231	70	55	46	31
50	974	415	510	220	68	64	46	25
52	1450	418	515	249	47	55	50	63
54	1350	466	542	255	41	51	50	52
57	979	410	475	218	56	65	50	29
59	1074	436	495	222	43	55	58	57
61	542	362	435	188	54	51	50	35
64	951	359	355	166	59	51	56	43
65	1250	455	534	255	54	55	47	27
68	851	350	437	149	45	51	56	51
71	1061	410	495	212	58	51	52	43
73	1456	418	577	243	58	55	54	43
75	595	385	525	241	52	55	45	32
78	1435	301	557	249	54	55	52	35
80	1451	350	572	245	52	51	21	31
82	593	374	583	243	59	55	50	37
85	1475	54	545	249	59	55	50	35
87	1035	462	527	256	51	55	52	33

Table D1.1: Phase II data for COD and sCOD cont.

OPERATIONAL DAY	INFLUENT AVERAGE COD (mg/L)	INFLUENT AVERAGE sCOD (mg/L)	ANOXIC EFFLUENT COD (mg/L)	ANOXIC EFFLUENT sCOD (mg/L)	AERATION EFFLUENT sCOD (mg/L)	RAS sCOD = INFL. DIGESTER (mg/L)	CLARIFIER EFFLUENT sCOD (mg/L)	CLARIFIER EFFLUENT COD (mg/L)
85	1475	541	545	249	33	29	20	36
87	1326	462	527	256	31	28	22	38
89	1270	423	612	282	49	39	32	59
92	1593	564	702	260	41	36	30	63
94	1091	496	616	229	36	27	21	45
96	1563	588	743	271	46	37	30	63
99	1453	584	715	269	43	37	28	48
101	1022	500	680	258	39	35	27	42
103	1479	566	706	264	43	38	29	46
106	1463	567	706	264	40	37	29	39
108	963	471	684	254	39	35	26	44
110	1473	584	725	269	41	38	30	41
113	1363	551	669	246	41	35	28	44
115	919	452	634	242	39	33	25	41
117	715	377	616	238	38	34	28	46
120	1580	559	699	261	42	34	27	47
122	1111	502	676	250	39	35	26	45
124	1490	591	707	269	43	37	30	48
127	1471	583	705	267	39	35	26	47
129	1045	489	669	262	44	38	30	51
131	1443	558	692	267	41	36	28	49
134	1040	412	763	277	48	42	32	68
136	1018	403	693	256	56	50	36	86
138	1255	474	745	285	53	45	34	77
141	821	393	659	250	59	50	43	78
143	1375	540	699	281	46	36	31	66
145	1550	570	720	296	51	42	35	71
148	974	429	702	233	48	41	32	64
150	1240	492	718	259	51	45	35	70
152	1263	503	713	269	48	44	33	66
155	1394	535	746	269	53	48	37	70
157	1173	429	724	261	50	45	35	67
159	1301	481	738	270	53	47	37	71
162	1433	515	748	274	50	45	35	69

Table D1.2: Phase III data for COD and sCOD

OPERATIONAL DAY	INFLUENT AVERAGE **COD** (mg/L)	INFLUENT AVERAGE **sCOD** (mg/L)	ANOXIC EFFLUENT **COD** (mg/L)	ANOXIC EFFLUENT **sCOD** (mg/L)	AERATION EFFLUENT **sCOD** (mg/L)	RAS **sCOD** = INFL. DIGESTER (mg/L)	CLARIFIER EFFLUENT **sCOD** (mg/L)	CLARIFIER EFFLUENT **COD** (mg/L)
162	1433	515	748	274	50	45	35	63
164	1082	413	600	246	32	24	19	54
166	930	386	573	235	25	17	14	57
169	1032	413	611	250	30	18	15	45
171	952	399	572	235	24	15	13	44
173	1021	430	618	230	21	20	19	40
176	1040	413	593	233	19	17	16	21
178	985	413	594	235	21	16	15	20
180	1013	420	603	231	19	20	19	23
183	1001	444	591	231	19	18	16	21
185	955	427	575	232	21	19	18	19
187	1028	442	715	281	36	34	28	56
190	1007	424	633	250	35	26	21	48
192	990	408	608	240	27	20	16	33
194	987	413	616	244	32	19	16	33
197	1014	404	627	247	31	27	22	42
199	992	416	611	241	22	22	20	27
201	1005	398	593	241	23	21	19	29
204	985	411	614	242	23	21	20	24
206	969	397	592	240	22	22	20	30
208	1024	404	570	242	23	21	19	26
211	1050	420	674	267	43	31	27	71
213	1033	406	650	258	33	23	21	55
215	1003	405	581	231	40	25	22	42
218	1020	457	693	277	37	31	26	57
220	1016	437	658	263	28	26	25	33
222	1072	438	680	253	25	24	21	35
225	1017	467	677	255	24	25	22	31
227	1011	426	659	257	27	27	23	29
229	1015	444	660	253	25	25	21	32
232	1026	439	648	254	27	24	23	36
234	1075	460	746	291	46	39	39	72
236	1029	446	699	273	40	31	27	77
239	1037	445	632	247	34	26	23	51
241	1065	471	711	278	41	39	32	50
243	1022	445	588	241	28	25	21	35
246	1024	423	681	266	29	26	24	41
248	1015	436	668	268	28	24	23	38
250	1034	416	658	253	30	29	25	44
253	1023	439	675	268	28	26	24	41
255	1037	423	662	263	30	30	27	43
257	1036	416	762	298	46	44	42	70
260	1037	445	707	284	33	31	28	85
262	1000	438	658	265	28	26	22	59
264	1030	455	684	275	41	39	31	58
267	1013	412	636	266	26	24	21	43
269	1040	412	651	279	33	31	33	49
271	1014	426	660	276	33	33	28	45
274	1002	385	636	277	33	32	32	53
276	1032	417	660	282	31	32	30	47
278	1013	397	642	278	33	32	28	50

Table D1.3: Phase II data for BOD_5

DATE	SAMPLING DAYS	INFLUENT BOD_5	EFFLUENT BOD_5	INFLUENT COD/BOD5 RATIO	EFFLUENT COD/BOD_5 RATIO
08/06/15	1	981	108	0.8	0.3
10/06/15	3				
12/06/15	5	854	127	0.6	0.3
15/06/15	8				
17/06/15	10				
19/06/15	12	933	71	0.8	0.3
22/06/15	15				
24/06/15	17				
26/06/15	19	695	14	0.9	0.3
29/06/15	22				
01/07/15	24				
03/07/15	26	686	5	0.6	0.2
06/07/15	29				
08/07/15	31	689	8	0.3	0.3
10/07/15	33	937	8	0.6	0.3
13/07/15	36				
15/07/15	38				
17/07/15	40	822	7	0.5	0.2
20/07/15	43				
22/07/15	45				
24/07/15	47	927	10	0.6	0.3
27/07/15	50				
29/07/15	52				
31/07/15	54	628	18	0.4	0.3
03/08/15	57				
05/08/15	59				
07/08/15	61	689	7	0.7	0.2
10/08/15	64				
12/08/15	66				
14/08/15	68	711	13	1.0	0.2
17/08/15	71				
19/08/15	73				
21/08/15	75	809	7	0.9	0.2
24/08/15	78				
26/08/15	80				
28/08/15	82	729	5	0.9	0.1
31/08/15	85				
02/09/15	87				

Table D1.3: Phase II data for BOD_5

DATE	SAMPLING DAYS	INFLUENT BOD_5	EFFLUENT BOD_5	INFLUENT COD/BOD5 RATIO	EFFLUENT COD/BOD_5 RATIO
31/08/15	85				
02/09/15	87				
04/09/15	89	793	16	0.6	0.3
07/09/15	92				
09/09/15	94				
11/09/15	96	866	17	0.5	0.3
14/09/15	99				
16/09/15	101				
18/09/15	103	782	15	0.5	0.3
21/09/15	106				
23/09/15	108				
25/09/15	110	810	24	0.5	0.6
28/09/15	113				
30/09/15	115				
02/10/15	117	662	12	0.7	0.3
05/10/15	120				
07/10/15	122				
09/10/15	124	804	17	0.5	0.4
12/10/15	127				
14/10/15	129				
16/10/15	131				
19/10/15	134				
21/10/15	136				
23/10/15	138	807	20	0.6	0.3
26/10/15	141				
28/10/15	143				
30/10/15	145	845	17	0.5	0.2
02/11/15	148				
04/11/15	150				
06/11/15	152	846	19	0.6	0.3
09/11/15	155				
11/11/15	157				
13/11/15	159	922	17	0.7	0.2
16/11/15	162	0	0	0.0	0.0

Table D1.3: Phase III data for BOD_5

DATE	SAMPLING DAYS	INFLUENT BOD_5	EFFLUENT BOD_5	INFLUENT COD/BOD5 RATIO	EFFLUENT COD/BOD_5 RATIO
16/11/15	162	0	0	0.0	0.0
18/11/15	164	0	0	0.0	0.0
20/11/15	166	570	15	0.6	0.3
23/11/15	169	0	0	0.0	0.0
25/11/15	171	0	0	0.0	0.0
27/11/15	173	516	13	0.5	0.3
30/11/15	176	0	0	0.0	0.0
02/12/15	178	0	0	0.0	0.0
04/12/15	180	483	3	0.5	0.1
07/12/15	183				
09/12/15	185				
11/12/15	187	514	10	0.5	0.2
14/12/15	190				
16/12/15	192				
18/12/15	194	628	11	0.6	0.3
21/12/15	197				
23/12/15	199				
25/12/15	201	661	4	0.6	0.1
28/12/15	204				
30/12/15	206				
01/01/16	208	645	9	0.6	0.4
04/01/16	211				
06/01/16	213				
08/01/16	215	635	17	0.7	0.4
11/01/16	218				
13/01/16	220				
15/01/16	222	644	6	0.6	0.2
18/01/16	225				
20/01/16	227				
22/01/16	229	570	10	0.6	0.3
25/01/16	232				
27/01/16	234				
29/01/16	236	598	24	0.6	0.3
01/02/16	239				
03/02/16	241				
05/02/16	243	613	10	0.6	0.3
08/02/16	246				
10/02/16	248				
12/02/16	250	542	14	0.5	0.3

Table D1.4: Phase II data for Total suspended solids

DATE	OPERATIONAL DAYS	TSS Influent (mg/L)	TSS Effluent (mg/L)
08/06/15	1	533	209
10/06/15	3	694	222
12/06/15	5	715	220
15/06/15	8	487	143
17/06/15	10	529	130
19/06/15	12	560	134
22/06/15	15	544	106
24/06/15	17	510	94
26/06/15	19	519	43
29/06/15	22	478	24
01/07/15	24	694	23
03/07/15	26	642	28
06/07/15	29	675	22
08/07/15	31	681	21
10/07/15	33	731	25
13/07/15	36	547	19
15/07/15	38	748	22
17/07/15	40	723	18
20/07/15	43	497	19
22/07/15	45	711	26
24/07/15	47	707	20
27/07/15	50	488	23
29/07/15	52	739	56
31/07/15	54	676	47
03/08/15	57	487	27
05/08/15	59	508	45
07/08/15	61	434	27
10/08/15	64	411	43
12/08/15	66	586	22
14/08/15	68	360	40
17/08/15	71	497	31
19/08/15	73	739	33
21/08/15	75	497	24
24/08/15	78	711	25
26/08/15	80	707	29
28/08/15	82	488	27
31/08/15	85	739	26
02/09/15	87	676	23
04/09/15	89	655	65
07/09/15	92	731	30
09/09/15	94	586	44
11/09/15	96	731	32
14/09/15	99	715	42
16/09/15	101	519	30
18/09/15	103	748	36
21/09/15	106	739	30
23/09/15	108	488	29
25/09/15	110	739	36
28/09/15	113	642	31
30/09/15	115	487	34
02/10/15	117	403	31
05/10/15	120	731	35
07/10/15	122	586	30
09/10/15	124	731	34
12/10/15	127	715	31
14/10/15	129	519	34
16/10/15	131	748	40
19/10/15	134	518	51
21/10/15	136	516	62
23/10/15	138	624	50
26/10/15	141	597	59
28/10/15	143	681	44
30/10/15	145	705	48
02/11/15	148	523	43
04/11/15	150	619	47
06/11/15	152	627	50
09/11/15	155	698	44
11/11/15	157	593	51
13/11/15	159	695	46
16/11/15	162	719	51

Table D1.4: Phase III data for Total suspended solids cont.

DATE	OPERATIONAL DAYS	TSS Influent (mg/L)	TSS Effluent (mg/L)
18/11/15	164	510	34
20/11/15	166	483	43
23/11/15	169	491	47
25/11/15	171	471	35
27/11/15	173	510	41
30/11/15	176	516	25
02/12/15	178	497	26
04/12/15	180	507	24
07/12/15	183	512	26
09/12/15	185	505	28
11/12/15	187	533	48
14/12/15	190	517	44
16/12/15	192	499	34
18/12/15	194	490	38
21/12/15	197	516	40
23/12/15	199	497	31
25/12/15	201	507	27
28/12/15	204	516	30
30/12/15	206	499	29
01/01/16	208	487	31
04/01/16	211	522	50
06/01/16	213	505	39
08/01/16	215	481	35
11/01/16	218	505	43
13/01/16	220	487	30
15/01/16	222	539	36
18/01/16	225	498	30
20/01/16	227	492	35
22/01/16	229	531	32
25/01/16	232	515	33
27/01/16	234	546	51
29/01/16	236	508	53
01/02/16	239	515	40
03/02/16	241	537	39
05/02/16	243	489	30
08/02/16	246	491	40
10/02/16	248	481	37
12/02/16	250	505	40
15/02/16	253	481	35
17/02/16	255	527	38
19/02/16	257	523	59
22/02/16	260	519	46
24/02/16	262	487	46
26/02/16	264	523	54
29/02/16	267	491	48
02/03/16	269	477	42
04/03/16	271	487	41
07/03/16	274	471	43
09/03/16	276	484	41
11/03/16	278	480	44

Table D1.5: Phase II data for Ammonia, Nitrate, Nitrite, TKN and TN

INFLUENT CONC., (mg/L)						EFFLUENT CONC., (mg/L)				
DATE	OPERATIONAL DAY	TKN	NH_4-N	NO_3-N	TN	TKN	NH_4-N	NO_3-N	NO_2-N	TN
08/06/2015	1	79.1	45.8	0.5	80.0	5.9	5.8	8.7	0.11	14.7
10/06/2015	3		54.0	0.7	84.7		7.7	8.2	0.00	16.0
12/06/2015	5	88.3	56.3	0.4	88.7	8.1	8.1	10.1	0.13	18.3
15/06/2015	8		40.4	0.4	65.0		4.0	9.3	0.00	13.7
17/06/2015	10		50.8	0.3	78.7		5.7	10.6	0.00	16.3
19/06/2015	12	78.0	50.2	0.5	78.7	6.6	6.5	11.7	0.04	18.3
22/06/2015	15		53.8	0.4	86.7		6.3	10.3	0.00	16.7
24/06/2015	17		39.1	0.1	64.3		2.9	11.5	0.00	14.3
26/06/2015	19	81.3	52.2	0.2	81.3	5.1	5.0	10.9	0.03	16.0
29/06/2015	22		46.1	0.3	74.0		2.0	11.3	0.00	13.3
01/07/2015	24		43.8	0.2	70.7		1.4	10.8	0.00	12.3
03/07/2015	26	75.4	46.4	0.4	76.5	1.6	1.5	11.7	0.10	13.3
06/07/2015	29		44.6	0.3	70.3		1.2	11.0	0.00	12.3
08/07/2015	31		46.1	0.4	73.5		1.5	11.5	0.00	13.0
10/07/2015	33	71.5	46.8	0.5	71.0	1.4	1.3	10.9	0.07	12.3
13/07/2015	36		49.2	0.4	79.0		1.6	10.6	0.00	12.3
15/07/2015	38		46.3	0.5	74.7		1.5	11.2	0.00	12.7
17/07/2015	40	72.6	46.3	0.2	71.3	1.7	1.6	10.3	0.00	12.0
20/07/2015	43		46.0	0.3	73.7		1.4	10.9	0.00	12.3
22/07/2015	45		44.3	0.4	71.5		1.3	10.6	0.00	12.0
24/07/2015	47	71.6	45.1	0.5	71.3	1.7	1.6	10.6	0.05	12.3
27/07/2015	50		48.4	0.3	78.0		1.7	10.9	0.00	12.7
29/07/2015	52		40.8	0.6	65.7		1.2	11.8	0.00	13.0
31/07/2015	54	64.0	40.3	0.4	64.7	1.2	1.1	12.1	0.04	13.3
03/08/2015	57		49.3	0.4	80.0		1.6	13.3	0.00	15.0
05/08/2015	59		46.5	0.2	75.5		1.6	10.7	0.00	12.3
07/08/2015	61	71.1	45.4	0.1	70.5	1.5	1.4	11.5	0.02	13.0
10/08/2015	64		44.3	0.5	70.5		1.5	11.1	0.00	12.7
12/08/2015	66		44.8	0.4	72.0		1.5	11.8	0.00	13.3
14/08/2015	68	68.2	43.9	0.3	70.0	1.5	1.5	10.5	0.02	12.0
17/08/2015	71		49.8	0.3	80.0		1.6	11.3	0.00	13.0
19/08/2015	73		49.0	0.3	79.5		1.4	14.1	0.00	15.7
21/08/2015	75	77.3	50.8	0.3	77.7	1.7	1.7	12.8	0.10	14.7
24/08/2015	78		49.6	0.4	80.0		1.5	11.1	0.00	12.7
26/08/2015	80		49.3	0.5	78.3		1.5	10.4	0.00	12.0
28/08/2015	82	81.9	52.1	0.3	82.0	1.7	1.7	10.5	0.09	12.3
31/08/2015	85		56.0	0.3	88.3		1.7	10.9	0.00	12.7
02/09/2015	87		56.3	0.3	89.7		1.7	11.2	0.00	13.0

Table D1.5: Phase II data for Ammonia, Nitrate, Nitrite, TKN and TN cont.

INFLUENT CONC., (mg/L)						EFFLUENT CONC., (mg/L)				
DATE	OPERATIONAL DAY	TKN	NH_3-N	NO_3-N	TN	TKN	NH_3-N	NO_3-N	NO_2-N	TN
31/08/2015	85		56.0	0.3	88.3		1.7	10.9	0.00	12.7
02/09/2015	87		56.3	0.3	89.7		1.7	11.2	0.00	13.0
04/09/2015	89	87.3	54.3	0.6	88.3	2.6	2.5	12.0	0.05	14.7
07/09/2015	92		58.4	0.6	92.3		3.1	12.5	0.00	15.7
09/09/2015	94		51.8	0.3	82.7		2.1	14.2	0.00	16.3
11/09/2015	96	87.3	56.5	0.7	87.7	2.6	2.5	11.7	0.08	14.3
14/09/2015	99		55.8	0.4	87.3		2.7	12.8	0.00	15.7
16/09/2015	101		51.5	0.5	82.7		2.2	12.4	0.00	14.7
18/09/2015	103	88.6	56.7	0.5	89.3	2.2	2.2	12.3	0.09	14.7
21/09/2015	106		55.8	0.5	88.7		2.3	12.6	0.00	15.0
23/09/2015	108		49.1	0.3	77.7		2.1	12.5	0.00	14.7
25/09/2015	110	90.2	56.7	0.3	90.7	2.3	2.2	12.7	0.07	15.0
28/09/2015	113	87.8	55.3	0.3	88.0	2.4	2.3	17.5	0.08	20.0
30/09/2015	115	82.0	51.3	0.2	82.3	2.4	2.3	16.5	0.09	19.0
02/10/2015	117	81.6	51.3	0.4	82.0	2.2	2.2	17.4	0.05	19.7
05/10/2015	120	87.6	55.0	0.1	88.0	2.4	2.3	17.5	0.09	20.0
07/10/2015	122	73.6	46.3	0.3	74.0	2.1	2.1	16.8	0.08	19.0
09/10/2015	124	89.0	56.5	0.4	89.3	2.3	2.3	17.2	0.09	19.7
12/10/2015	127	88.5	56.1	0.3	88.7	2.4	2.3	17.3	0.03	19.7
14/10/2015	129	80.4	50.5	0.3	81.7	2.2	2.1	17.1	0.09	19.3
16/10/2015	131	83.6	55.1	0.5	84.3	2.8	2.7	17.2	0.06	20.0
19/10/2015	134	94.5	59.3	0.3	94.7	3.3	3.2	13.3	0.06	16.7
21/10/2015	136		52.6	0.5	84.7		2.8	14.8	0.00	17.7
23/10/2015	138	81.9	54.3	0.6	82.7	3.0	2.9	11.9	0.10	15.0
26/10/2015	141		44.8	0.4	70.7		2.2	13.5	0.00	15.7
28/10/2015	143		56.8	0.4	91.3		3.0	14.3	0.00	17.3
30/10/2015	145	91.4	57.6	0.6	91.7	3.0	2.9	13.9	0.10	17.0
02/11/2015	148		53.0	0.4	85.3		2.8	13.8	0.00	16.7
04/11/2015	150		48.4	0.6	76.3		2.8	13.5	0.00	16.3
06/11/2015	152	88.1	55.3	0.4	88.7	2.9	2.9	14.4	0.08	17.3
09/11/2015	155		56.4	0.6	90.7		2.9	14.1	0.00	17.0
11/11/2015	157		57.1	0.3	91.7		2.9	13.7	0.00	16.7
13/11/2015	159	82.3	51.1	0.5	82.7	2.9	2.9	13.7	0.06	16.7
16/11/2015	162		56.8	0.5	89.7		2.8	14.3	0.00	17.3

Table D1.6: Phase III data for Ammonia, Nitrate, Nitrite, TKN and TN

INFLUENT CONC., (mg/L)						EFFLUENT CONC., (mg/L)				
DATE	OPERATIONAL DAY	TKN	NH_4-N	NO_3-N	TN	TKN	NH_4-N	NO_3-N	NO_2-N	TN
16/11/2015	162		56.8	0.5	89.7		2.8	14.3	0.00	17.3
18/11/2015	164		53.0	0.6	85.3		3.5	14.4	0.00	17.0
20/11/2015	166	81.3	54.6	0.3	81.7	3.2	2.3	14.1	0.08	17.3
23/11/2015	169		51.8	0.5	83.3		2.5	15.8	0.00	19.7
25/11/2015	171		52.8	0.6	85.3		1.9	14.4	0.00	16.7
27/11/2015	173	79.1	52.3	0.5	80.3	2.4	2.2	14.1	0.09	17.0
30/11/2015	176		52.9	0.4	85.3		2.1	13.8	0.00	16.3
02/12/2015	178		53.3	0.6	86.7		2.3	13.4	0.00	16.0
04/12/2015	180	87.5	54.0	0.3	87.3	2.4	2.3	14.0	0.05	17.0
07/12/2015	183		53.1	0.5	86.7		2.5	13.9	0.00	16.7
09/12/2015	185		52.4	0.2	83.7		2.3	13.4	0.00	16.3
11/12/2015	187	80.9	51.3	0.1	79.7	3.4	3.2	17.6	0.05	21.7
14/12/2015	190		55.4	0.2	88.7		3.1	15.4	0.00	19.0
16/12/2015	192		57.0	0.3	92.3		4.2	16.4	0.00	21.3
18/12/2015	194	86.7	53.0	0.5	85.0	3.3	3.2	17.5	0.06	21.3
21/12/2015	197		51.8	0.3	83.0		2.7	14.7	0.00	18.3
23/12/2015	199		53.0	0.6	84.7		3.2	14.4	0.00	18.7
25/12/2015	201	85.5	53.6	0.5	86.7	3.4	3.1	14.9	0.08	19.0
28/12/2015	204		54.3	0.3	86.7		3.2	14.6	0.00	18.7
30/12/2015	206		53.4	0.4	85.3		3.3	14.9	0.00	19.0
01/01/2016	208	83.3	53.0	0.3	85.0	3.5	3.2	14.5	0.01	19.3
04/01/2016	211		56.0	0.4	79.3		3.3	18.3	0.00	22.3
06/01/2016	213		52.6	0.3	88.7		3.2	16.3	0.00	20.0
08/01/2016	215	85.4	53.1	0.6	85.7	3.6	3.4	18.8	0.08	23.3
11/01/2016	218		51.8	0.6	79.5		2.9	16.5	0.00	20.7
13/01/2016	220		53.0	0.3	85.0		3.1	15.3	0.00	19.3
15/01/2016	222	91.1	56.5	0.5	92.0	3.7	3.6	15.5	0.04	20.3
18/01/2016	225		49.0	0.6	78.7		3.4	15.3	0.00	19.3
20/01/2016	227		52.8	0.5	85.0		3.5	15.2	0.00	19.3
22/01/2016	229	82.6	51.6	0.4	83.0	3.4	3.3	15.4	0.03	19.3
25/01/2016	232		50.4	0.6	81.3		3.4	15.6	0.00	20.3
27/01/2016	234		51.1	0.3	80.7		3.8	19.6	0.00	24.0
29/01/2016	236	89.3	56.5	0.5	89.7	4.1	4.0	18.1	0.03	23.0
01/02/2016	239		55.8	0.2	88.7		3.2	15.9	0.00	20.0
03/02/2016	241		52.6	0.1	81.7		3.1	18.9	0.00	22.7
05/02/2016	243	85.3	54.1	0.2	85.7	4.1	4.0	16.0	0.07	21.0
08/02/2016	246		55.3	0.3	83.7		4.1	16.4	0.00	21.7
10/02/2016	248		49.0	0.5	79.0		3.9	16.8	0.00	21.3
12/02/2016	250	82.2	51.8	0.3	82.7	4.2	4.1	16.2	0.00	21.3
15/02/2016	253		48.3	0.6	79.7		4.0	16.8	0.00	21.7
17/02/2016	255		51.8	0.5	82.3		4.3	16.6	0.00	21.3
19/02/2016	257	88.2	56.6	0.3	88.7	5.2	5.1	20.4	0.06	26.3
22/02/2016	260		48.4	0.4	78.7		4.6	17.4	0.00	23.7
24/02/2016	262		52.6	0.3	87.3		5.0	19.5	0.00	25.0
26/02/2016	264	85.6	54.3	0.4	86.3	5.6	5.5	18.1	0.07	24.3
29/02/2016	267		50.5	0.3	81.0		5.1	18.8	0.00	24.7
02/03/2016	269		49.9	0.6	80.0		5.2	17.7	0.00	23.7
04/03/2016	271	83.7	51.8	0.2	84.0	5.3	5.2	18.8	0.07	24.7
07/03/2016	274		49.8	0.1	79.7		5.1	17.9	0.00	23.7
09/03/2016	276		50.1	0.2	80.7		5.2	17.7	0.00	23.3
11/03/2016	278	81.7	48.8	0.3	82.3	5.2	5.1	18.6	0.07	24.0

APPENDIX E

Table E1.1: Anoxic chamber denitrification performance

Day	Qin (Lpd)	IR	QRAS, Lpd	Qin * IR (Lpd)	QRAS Lpd	Qc (combined flow, L d)	ΔCOD ΔNO3	ANX-C Nitr Loadg (g d)	Nitrate conc into ANX-C (mg L)	Denitrn Effic, %	Specific denitrn Rate (mg NO3-N g VSS d)	Spec COD Rem Rate-ANX-C (mg COD mg VSS d)	Spec COD Rem Rate-AER-C (mg COD mg VSS d)
1	45	3	0 $	135	36	216	22 1	0 11	26 6	99 5	94	0 451	0 146
3	45	3	0 $	135	36	216	30 3	0 11	25 $	99 4	99	0 626	0 201
5	45	3	0 $	135	36	216	28 1	0 12	29 1	99 4	118	0 690	0 208
8	45	3	0 $	135	36	216	16 3	0 11	27 0	99 0	119	0 403	0 118
10	45	3	0 $	135	36	216	17 $	0 12	30 0	99 3	125	0 461	0 140
12	45	3	0 $	135	36	216	17 9	0 12	27 7	99 4	105	0 391	0 126
15	45	3	0 $	135	36	216	15 5	0 12	29 5	99 4	106	0 340	0 103
17	45	3	0 $	135	36	216	15 7	0 13	31 7	99 5	106	0 347	0 104
19	45	3	0 $	135	36	216	15 6	0 13	30 8	99 2	86	0 279	0 099
22	45	3	0 $	135	36	216	13 3	0 13	31 6	99 5	84	0 234	0 081
24	45	3	0 $	135	36	216	25 2	0 13	32 4	98 8	74	0 387	0 138
26	45	3	0 $	135	36	216	17 $	0 13	32 0	98 6	67	0 250	0 086
29	45	3	0 $	135	36	216	24 1	0 13	32 1	98 3	68	0 342	0 105
31	45	3	0 $	135	36	216	17 2	0 13	31 9	98 7	66	0 237	0 072
33	45	6	0 $	270	23	351	30 $	0 24	32 2	98 5	103	0 405	0 134
36	45	6	0 $	270	23	351	18 3	0 24	31 7	98 5	109	0 255	0 072
38	45	6	0 $	270	23	351	27 3	0 25	32 5	98 7	101	0 353	0 118
40	45	6	0 $	270	23	351	27 5	0 24	31 4	98 5	112	0 396	0 113
43	45	6	0 $	270	23	351	14 3	0 24	32 1	98 7	105	0 192	0 062
45	45	6	0 $	270	23	351	27 1	0 24	31 4	98 6	106	0 367	0 105
47	45	6	0 $	270	23	351	26 9	0 24	31 7	98 6	102	0 353	0 118
50	45	6	0 $	270	23	351	14 $	0 24	31 $	98 6	104	0 197	0 061
52	45	6	0 $	270	23	351	25 2	0 26	34 7	98 $	98	0 316	0 122

Table E1.1: Anoxic chamber denitrification performance cont.

Day	Qin (Lpd)	IR	QRAS, Lpd	Qin * IR (Lpd)	QRAS, Lpd	Qc (combined flow, L/d)	ΔCOD ΔNO3	ANX-C Nitr. Loadg (g/d)	Nitrate conc. Into ANX-C (mg/L)	Denitri. Effic, %	Specific denitri. Rate (mg NO3-N/g. VSS.d)	Spec. COD Rem. Rate-ANX-C. (mg COD/mg. VSS.d)	Spec. COD Rem. Rate-AER-C. (mg COD/mg. VSS.d)
57	45	6	0.8	270	23	351	14.6	0.26	34.9	98.8	102	0.190	0.063
59	45	6	0.8	270	23	351	18.3	0.24	32.1	98.6	99	0.231	0.069
61	45	6	0.8	270	23	351	15.5	0.25	32.8	98.9	94	0.187	0.069
64	45	6	0.8	270	23	351	11.4	0.24	32.1	98.7	94	0.138	0.047
66	45	6	0.8	270	23	351	21.9	0.24	31.8	98.5	93	0.262	0.098
68	45	6	0.8	270	23	351	14.1	0.24	31.8	98.6	92	0.167	0.060
71	45	6	0.8	270	23	351	16.6	0.24	32.3	97.9	89	0.189	0.067
73	45	6	0.8	270	23	351	26.3	0.26	34.5	98.3	96	0.323	0.118
75	45	6	0.8	270	23	351	14.2	0.25	33.5	98.7	95	0.173	0.058
78	45	6	0.8	270	23	351	27.8	0.24	31.9	98.6	91	0.323	0.109
80	45	6	0.8	270	23	351	27.2	0.24	32.2	98.7	91	0.318	0.117
82	45	6	0.8	270	23	351	14.7	0.24	31.8	98.6	88	0.166	0.061
85	45	6	0.8	270	23	351	29.4	0.24	32.0	98.6	92	0.348	0.127
87	45	6	0.8	270	23	351	25.1	0.24	32.3	98.6	93	0.298	0.097
89	70	6	0.5	420	35	525	18.9	0.40	35.2	98.8	122	0.308	0.122
92	70	6	0.5	420	35	525	25.1	0.41	36.0	98.7	126	0.422	0.152
94	70	6	0.5	420	35	525	13.0	0.42	36.8	98.8	146	0.254	0.084
96	70	6	0.5	420	35	525	23.5	0.40	35.3	98.9	156	0.5	0.1
99	70	6	0.5	420	35	525	20.7	0.41	36.1	98.8	148	0.4	0.1
101	70	6	0.5	420	35	525	9.7	0.40	35.6	98.8	158	0.21	0.06
103	70	6	0.5	420	35	525	21.8	0.41	36.0	98.7	153	0.44	0.15
106	70	6	0.5	420	35	525	21.3	0.41	36.1	98.7	148	0.42	0.14
108	70	6	0.5	420	35	525	7.9	0.41	35.9	98.8	153	0.16	0.06

Table E1.1: Anoxic chamber denitrification performance cont.

Day	Qin (Lpd)	IR	QRAS, Lpd	Qin * IR (Lpd)	QRAS, Lpd	Qc (combined flow, L/d)	ΔCOD/ΔNO3	ANX-C Nitr. Loadg (g/d)	Nitrate conc. into ANX-C (mg/L)	Denitri. Effic, %	Specific denitri. Rate (mg NO3-N/g. VSS .d)	Spec. COD Rem. Rate-ANX-C. (mg COD/mg. VSS .d)	Spec. COD Rem. Rate-AER-C. (mg COD/mg. VSS .d)
113	70	3	0.5	210	35	315	17.6	0.24	39.9	98.9	101	0.39	0.13
115	70	3	0.5	210	35	315	7.6	0.23	38.1	98.5	92	0.15	0.06
117	70	3	0.5	210	35	315	2.5	0.24	39.7	98.9	102	0.06	0.02
120	70	3	0.5	210	35	315	22.2	0.24	40.0	98.3	101	0.50	0.17
122	70	3	0.5	210	35	315	11.4	0.23	38.7	98.7	97	0.25	0.09
124	70	3	0.5	210	35	315	20.0	0.24	39.6	99.0	99	0.44	0.14
127	70	3	0.5	210	35	315	19.5	0.24	39.7	98.9	97	0.42	0.15
129	70	3	0.5	210	35	315	9.6	0.24	39.6	98.9	105	0.22	0.07
131	70	3	0.5	210	35	315	19.0	0.24	40.0	98.8	104	0.44	0.15
134	100	6	0.5	600	50	750	7.2	0.63	39.1	98.9	152	0.35	0.07
136	100	6	0.5	600	50	750	8.7	0.61	37.8	98.8	150	0.17	0.08
138	100	6	0.5	600	50	750	13.4	0.62	38.5	98.9	145	0.26	0.12
141	100	6	0.5	600	50	750	4.6	0.62	38.2	98.7	155	0.09	0.04
143	100	6	0.5	600	50	750	17.6	0.63	38.8	98.8	164	0.38	0.16
145	100	6	0.5	600	50	750	21.6	0.63	38.9	98.8	164	0.47	0.21
148	100	6	0.5	600	50	750	7.1	0.63	38.9	98.8	172	0.16	0.07
150	100	6	0.5	600	50	750	13.4	0.64	39.4	98.5	167	0.30	0.13
152	100	6	0.5	600	50	750	14.5	0.62	38.5	98.5	168	0.32	0.14
155	100	6	0.5	600	50	750	16.6	0.64	39.5	98.7	171	0.38	0.15
157	100	6	0.5	600	50	750	11.8	0.62	38.6	98.8	164	0.26	0.11
159	100	6	0.5	600	50	750	14.5	0.63	39.2	98.7	173	0.34	0.12
162	100	6	0.5	600	50	750	17.8	0.63	39.1	98.6	165	0.39	0.15
164	100	6	0.5	600	50	750	12.0	0.66	40.8	98.7	259	0.41	0.11

Table E1.1: Anoxic chamber denitrification performance cont.

Day	Qin (Lpd)	IR	QRAS, Lpd	Qin * IR (Lpd)	QRAS, Lpd	Qc (combined flow, L/d)	ΔCOD ΔNO3	ANX-C Nitr Loadg (g/d)	Nitrate conc. Into ANX-C (mg/L)	Denitri. Effic, %	Specific denitri. Rate (mg NO_3-N/g. VSS .d)	Spec. COD Rem. Rate-ANX-C. (mg COD/mg. VSS .d)	Spec. COD Rem. Rate-AER-C. (mg COD/mg. VSS .d)
169	100	6	0.5	600	50	750	10.9	0.65	39.2	98.5	261	0.38	0.10
171	100	6	0.5	600	50	750	9.8	0.64	39.3	98.7	257	0.34	0.09
173	100	6	0.5	600	50	750	11.1	0.60	36.9	98.4	259	0.38	0.11
176	100	6	0.5	600	50	750	11.5	0.65	40.2	98.9	305	0.46	0.12
178	100	6	0.5	600	50	750	10.6	0.60	37.4	98.9	277	0.39	0.10
180	100	6	0.5	600	50	750	10.6	0.63	39.0	98.8	292	0.41	0.11
183	100	6	0.5	600	50	750	10.7	0.63	38.7	98.8	303	0.43	0.10
185	100	6	0.5	600	50	750	10.3	0.60	37.4	98.7	290	0.40	0.10
187	110	6	0.5	660	55	825	7.5	0.75	42.3	98.6	280	0.28	0.08
190	110	6	0.5	660	55	825	9.0	0.75	42.0	98.8	292	0.35	0.10
192	110	6	0.5	660	55	825	9.8	0.70	39.4	98.5	273	0.36	0.11
194	110	6	0.5	660	55	825	8.6	0.77	43.5	98.9	271	0.31	0.09
197	110	6	0.5	660	55	825	10.0	0.70	39.3	98.6	251	0.35	0.12
199	110	6	0.5	660	55	825	9.9	0.69	38.9	98.7	286	0.38	0.11
201	110	6	0.5	660	55	825	10.3	0.72	40.3	98.8	285	0.39	0.11
204	110	6	0.5	660	55	825	9.6	0.70	39.1	98.7	278	0.36	0.11
206	110	6	0.5	660	55	825	9.5	0.71	40.1	98.8	280	0.36	0.11
208	110	6	0.5	660	55	825	11.6	0.70	39.7	98.7	285	0.44	0.13
211	120	6	0.5	720	60	900	8.6	0.87	48.0	98.5	255	0.29	0.09
213	120	6	0.5	720	60	900	9.2	0.82	42.2	98.5	226	0.28	0.12
215	120	6	0.5	720	60	900	9.7	0.85	45.9	98.6	263	0.34	0.12
218	120	6	0.5	720	60	900	8.0	0.79	40.6	98.8	237	0.25	0.09
220	120	6	0.5	720	60	900	8.8	0.80	41.5	98.7	240	0.28	0.11

Table E1.1: Anoxic chamber denitrification performance cont.

Day	Qin (Lpd)	IR	QRAS, Lpd	Qin * IR (Lpd)	QRAS, Lpd	Qc (combined flow, L/d)	ΔCOD/ΔNO3	ANX-C Nitr. Loadg (g/d)	Nitrate conc. into ANX-C (mg/L)	Denitri. Effic, %	Specific denitri. Rate (mg NO3-N/g. VSS.d)	Spec. COD Rem Rate-ANX-C. (mg COD/mg. VSS.d)	Spec. COD Rem. Rate-AER-C. (mg COD/mg. VSS.d)
232	120	6	0.5	720	60	900	9.1	0.82	42.0	98.7	245	0.30	0.13
234	135	6	0.5	810	68	1013	7.3	0.99	45.4	98.5	245	0.24	0.09
236	135	6	0.5	810	68	1013	7.5	0.98	44.8	98.7	239	0.24	0.10
239	135	6	0.5	810	68	1013	9.3	0.96	43.9	98.6	246	0.31	0.14
241	135	6	0.5	810	68	1013	7.8	1.00	45.8	99.0	242	0.25	0.11
243	135	6	0.5	810	68	1013	10.7	0.90	41.2	98.9	225	0.32	0.16
245	135	6	0.5	810	68	1013	8.3	0.91	41.9	98.9	236	0.26	0.11
248	135	6	0.5	810	68	1013	8.2	0.93	42.7	98.9	242	0.26	0.12
250	135	6	0.5	810	68	1013	9.1	0.92	42.0	98.5	234	0.28	0.14
253	135	6	0.5	810	68	1013	8.1	0.95	43.5	99.0	234	0.25	0.12
255	135	6	0.5	810	68	1013	8.6	0.94	43.1	98.9	245	0.29	0.13
257	150	6	0.5	900	75	1125	7.4	1.10	45.5	98.6	235	0.23	0.11
260	150	6	0.5	900	75	1125	6.9	1.17	48.3	98.8	266	0.24	0.11
262	150	6	0.5	900	75	1125	7.0	1.19	49.2	98.9	257	0.24	0.11
264	150	6	0.5	900	75	1125	7.4	1.15	47.4	99.0	254	0.25	0.13
267	150	6	0.5	900	75	1125	8.9	1.16	47.7	99.0	259	0.27	0.13
269	150	6	0.5	900	75	1125	8.6	1.11	45.7	98.9	245	0.28	0.14
271	150	6	0.5	900	75	1125	7.8	1.11	45.9	98.9	250	0.26	0.13
274	150	6	0.5	900	75	1125	7.9	1.14	47.1	99.0	265	0.28	0.14
276	150	6	0.5	900	75	1125	8.2	1.11	45.7	98.9	249	0.27	0.14
278	150	6	0.5	900	75	1125	7.9	1.15	47.6	99.0	256	0.27	0.14

APPENDIX F
PUBLICATIONS

Journals:

1. **N. Aminu**, S.R.M. Kutty, M. H. Isa and I.U. Salihi, 'Influence Of Internal And Recycle On Nitrogen Removal In Compact Bioreactor', ***Research Journal of Applied Science, Engineering & Technology*** (ISSN: 20407459, 20407467). Maxwell Science Publication Corp.

Book Chapter:

1. Shamsul Rahman Mohamed Kutty, **Nasiru Aminu**, Mohamed Hasnain Isa, 'Compact Bioreactor Treatment System for Malaysian Municipal Sewage, In Physical, Chemical and Biological Treatment Processes for Water and Wastewater, Chemical Engineering and Technology', (ISBN: 978-1-63483-405-6) (e-Book). ***Nova Science Publishers***, Inc., New York, USA (2015).

Conferences:

1. Shamsul Rahman Bn Mohamed Kutty, Mohamed Hasnain Isa, **Nasiru Aminu**, Ibrahim Umar Salihi and Henry Ezerie, 'Potential of Compact Extended Aeration Reactor (CEAR) as integrated system to biologically degrade municipal sewage according to Malaysia regulatory limits', ***Energy and Sustainability V. WIT Transactions on Ecology and the Environment, Vol 186 (2014).***

2. **Nasiru Aminu**, Shamsul Rahman Mohamed Kutty, Mohamed Hasnain Isa, 'Evaluation of Biokinetic Co-efficients for Carbon Oxidation in Suspended Growth Using Compact Bioreactor', International Postgraduate Conf, (9-10th Dec, 2015), published in ***Advances In Environmental Biology*** (ISSN-1995-0756) (EISSN-1998-1066), AENSI Journals. (2016).

3. S.R.M. Kutty, **N. Aminu**, M.H. Isa & I.U. Salihi, 'Application of integrated bioreactor system (i-SGBR) for simultaneous treatment of wastewater and excess sludge degradation', *3rd International Conference on Civil, Offshore and Environmental Engineering (ICCOEE 2016)*, World Engineering Science and Technology Congress (ESTCON), Universiti Teknologi PETRONAS, Malaysia.

(August, 2016), *Engineering Challenges for Sustainable Future– Zawawi (Ed.),*

APPENDIX G
PRODUCT PATENT

IP 2016701 as "Integrated suspended growth bioreactor System" (i-SGBR) for treatment of domestic, industrial wastewater and sludge degradation.

Patents Form No.5 PATENTS ACT 1983 REQUEST FOR SUBSTANTIVE EXAMINATION (Regulations 27(1) and 45(3)) To: The Registrar of Patents Patents Registration Office Kuala Lumpur, Malaysia	For Official Use APPLICATION NO: **PI 2016701409** Filing Date: **18/04/2016** Request received on: **18/04/2016** Fee received on: **18/04/2016** Amount: **RM1370** *Cheque/Postal Order/Money Order/Draft/Cash No: Date of mailing:
Please sunmit this Form in duplicate together with prescribed fee.	Applicant's or Agent's file reference:

I. IN THE MATTER OF:
Patent Application No: Filing Date: **18/04/2016**
Certificate Application No: Filing Date: **18/04/2016**

II. APPLICANT(S):

Name: **Universiti Teknologi Petronas**
Address: **Bandar Seri Iskandar, 31750 Tronoh PERAK MALAYSIA**

III. REQUEST:
The applicant(s) request(s) the Registrar to refer the patent application identified above to an Examiner for a substantive examination in accordance with Section 29A (1) of the Patent Act 1983.

IV. ADDITIONAL INFORMATION accompanies this Form:
Yes No √

V.SIGNATURE: **mail=account@adastra.com.my, cn=MOHANA MURALI KODIVEL, ou=Contact Number - 0322842281,ou=Identity Card / Passport No -##########, ou=Terms of use at www.msctrustgate.com/rpa (c)00, ou=Bahagian Teknologi Maklumat V2, o=Perbadanan Harta Intelek Malaysia, l="A-28-10 MENARA UOA BANGSAR, NO.5 JALAN BANGSAR UTAMA 1", st="59000 KUALA LUMPUR, WILAYAH PERSEKUTUAN", c=MY, CertSerialNo=2216e31ce4afd0990dda52aab89933e3|**

****(~~Applicant~~/Agent)** **18/04/2016** (Date)

If Agent, indicate Agent's Registration No.: **PA/2004/0141**

For Official Use
Date application received: **18/04/2016**

* Delete whichever does not apply
** Type name under signature and delete whichever does not apply

APPENDIX H

AWARDS

1. **Bronze Medal** - Integrated Bioreactor System (i-SGBR), Malaysia Technology Exhibition, 18-20th February 2016, Kuala Lumpur Malaysia.

2. **Gold medal** - Integrated Bioreactor System, 27th International Invention & Innovation Exhibition (**ITEX**), 12-14th May, 2016 at Kuala Lumpur, Malaysia.

MARS

MALAYSIAN ASSOCIATION OF RESEARCH SCIENTISTS

Bronze Medal

This Certificate of Award is presented to

Associate Professor Dr. Shamsul Rahman Mohamed Kutty
Associate Professor Dr. Mohamed Hasnain Isa
Nasiru Aminu

For the invention/innovation of

Integrated Suspended Growth Bioreactor (i-SGBR) for Malaysian Municipal Sewage and Industrial Wastewater

Invention & Innovation Awards 2016
Malaysia Technology Expo 2016
18 - 20 February 2016
Kuala Lumpur

(Dr. Wan Manshol bin W Zin)
President
Malaysian Association of Research Scientists

MINISTRY OF SCIENCE, TECHNOLOGY & INNOVATION

ITEX
MALAYSIA
International Invention & Innovation Exhibition

UNDER THE PATRONAGE OF THE MINISTER OF SCIENCE, TECHNOLOGY AND INNOVATION

MINDS

Certificate of Participation

This is to certify that

ASSOC PROF DR SHAMSUL RAHMAN B M KUTTY,
AP DR HASNAIN, NASIRU AMINU

UNIVERSITI TEKNOLOGI PETRONAS, MALAYSIA

showcased the invention

I-SGBR

at the

27TH INTERNATIONAL INVENTION, INNOVATION & TECHNOLOGY EXHIBITION 2016

12 – 14 MAY 2016
KUALA LUMPUR, MALAYSIA

Academician Emeritus Professor
Tan Sri Datuk Dr Augustine Ong Soon Hock
President
Malaysian Invention and Design Society